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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

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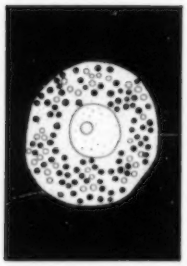

ACHIEVEMENTS IN SOVIET MICROBIOLOGY

Edited by A. A. Imshenetskii

Director of the Institute of Microbiology
of the Academy of Sciences of the USSR

TRANSLATED FROM RUSSIAN

(Original published for the Institute of Microbiology, Academy of Sciences of the USSR, by the Academy of Sciences Press, Moscow)

This concise survey of recent developments in (as well as projected directions of) Soviet microbiology is divided into four major sections:

I. Progress in General Microbiology, *A. A. Imshenetskii*
A broad review which enables the Western scientist to see clearly the direction in which Soviet microbiologists are making their studies.

II. Achievements of Soviet Microbiology in the Field of the Study of the Geological Activity of Microorganisms, *S. I. Kuznetsov*

In a field which has not been widely considered by Western scientists, this paper indicates great advances made in the USSR. The use of microorganisms in petroleum research and recovery are discussed, as are Soviet efforts to classify precisely and map out geologic or ecologic microbiological strata as they occur in the geologist's scheme. This article summarizes Soviet research into the relationships of various groups of microorganisms to geological – petroleum – inorganic substrates environments.

III. Forty Years of Technical Microbiology in the USSR, *V. N. Shaposhnikov, N. D. Ierusalimskii, and I. L. Rabotnova*

Noteworthy in this paper (which deals with fermentation microbiology, acetone-butanol, organic acids, riboflavin, antibiotics, etc.) is Shaposhnikov's description of disagreement in the USSR, as there has been in the West, on the biosynthesis of citric acid by various species of *Aspergillis.*

IV. Achievements in the Field of Soil Microbiology in the USSR, *E. N. Mishustin*

Soviet research in this area places particular emphasis on rhizosphere, root nodule, and nitrogen-fixing bacteria, and on the relationship between higher plants and associated microorganisms. Special attention is given to Vinogradskii's early description of auxotrophs and Krasilnikov's recent classification of actinomycetous microorganisms. Some comment is made on methodology, especially microbial counts and selection techniques.

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CHARACTERISTICS OF THE COURSE OF ACTIVE REST IN INDIVIDUALS OF ADVANCED AGE

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(Presented by Active Member of the AMN SSSR B. N. Man'kovskii)
Translated from Byulleten' Éksperimental' noi Biologii i Meditsiny, Vol. 51, No. 5,
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The central mechanism of the increase of work capacity under active rest is generally recognized at present [2, 3, 4, 6, 8, 12, 14, 17, 18]. The majority of investigators explain the increase of work capacity in the tired hand during the rest period, accompanied by the work of the other hand, as a result of the restorative role of inhibition which develops during the active rest period in the centers of the opposite group of muscles [2, 3, 4, 8, 14, 18].

The aim of the present work was to study the effect of active rest on the work capacity of individuals of advanced and old age. Comparison of the course of active and passive rest during this stage of life can be utilized for the working out of a rational regime of work and rest. In addition, such investigations may offer data for the characterization of changes in the restorative role of inhibition on the example of Sechenov phenomenon.

Our studies were based on numerous works which had come out from the I. P. Pavlov laboratory [1, 10, 11, 15, 16] and which point to the weakening of the internal inhibition process during the senescence of the organism, as well as on the premise developed by V. V. Frol'kis [19] on the weakening of the restorative role of inhibition while its protective character is preserved under certain functional changes.

METHOD OF INVESTIGATION

For the study of active rest we employed ergographs [7] which enabled us to evaluate the performance of flexors not of one finger only, but of the whole wrist. A special calculating device enabled us to evaluate the performed work.

The studies were carried out at the base of Kiev-Svyatoshinskii Home for the Aged on 12 females and 16 males of advanced age. Control observations were conducted on eight young men.

A total of 36 individuals was examined. According to age they were divided as follows: from 20 to 35 years—8, from 65 to 70—4, from 70 to 80—15, over 80—9 individuals.

A total of 186 observations were carried out. All observed individuals belonged to a group selected for study by the Institute of Gerontology and were thoroughly examined by the Institute clinicians. For control we used the data of the work of I. V. Muravov [7] which had been carried out according to the same method on 29 individuals aged from 18 to 28 years.

For the characterization of the effect of active and passive rest on work capacity we employed the following method of observation: the tested individual carried out some work for two minutes under the control of the metronome, by performing 34 or 45 work movements per minute. This rhythm was easily mastered. This work

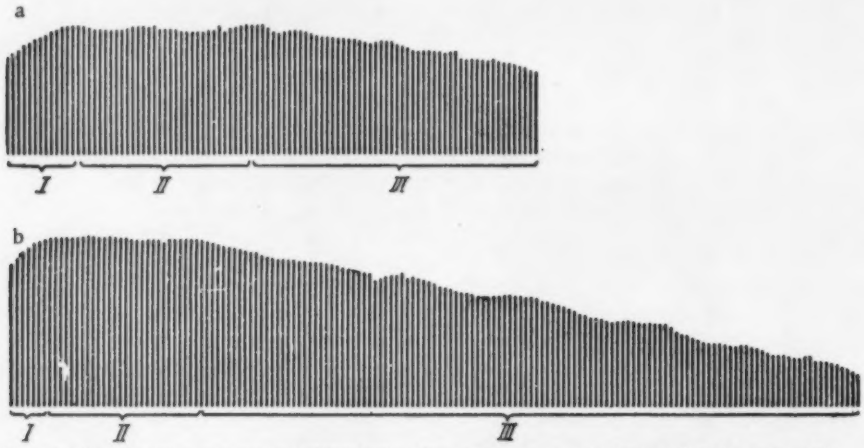
was followed by one minute pause during which, depending on the conditions of the test, an active or passive rest was suggested. Another two minute work followed, then a two minute pause followed by a three minute work.

In accordance with the law of power relations, three types of exertion were selected: slight, medium, and strong, the determination of which was effected on the basis of indicators of manual dynamometry. Slight exertions constituted 5-10% of maximum force of the wrist expressed in kilograms, medium—10-17%, strong—20%.

In establishing the duration of active rest, we employed the data of S. P. Narikashvili and Sh. A. Chakhnashvili [9] who had demonstrated in their special studies that an increase of active rest duration from 10 to 100-120 seconds progressively increases its restorative effect. Subsequent increase of active rest duration over 2-3 minutes does not contribute to the improvement of restoration of functions. On the basis of these data, we limited the duration of active rest to one minute.

RESULTS OF INVESTIGATION

In the course of our studies we observed a difference in the course of the fatigue curve and in the character of the ergogram in individuals of advanced age as compared to young people (see figure).



Ergographic fatigue curves in individuals of various ages. a) Tested S., 81 years old; b) tested B., 35 years old. I) "Breaking-in" period; II) period of stable work capacity; III) period of reduced work capacity.

As seen in the cited figure, in old people the breaking-in period is extended and the period of stable work capacity lasts longer than in young people, but the maximal level of contractions and the total work performed have a smaller range. Extension of the period of stable work capacity was observed also by O. F. Maksimova [5] and K. D. Predtechenskii [13].

The extension of the breaking-in period can be regarded as the result of slower engagement of the mechanisms of maximal work capacity in old people.

One characteristic feature is often observed in the ergographic studies of the aged. A number of those tested under conditions of high physical stress stated that they were unable to continue their work at this rate; however, after an interval of 10 to 20 seconds their work continued at a high level, and the tested individuals observed no disagreeable sensations. Apparently, in these cases some special mechanism prevents old people from developing an intense fatigue.

In the first series of tests we studied the effect of active and passive rest on the restoration of work capacity when the right hand was under medium physical stress. In these tests, the load on the left hand during active rest equalled 50% of a fatiguing load. The tested individual was given three work loads of two minutes each with one minute intervals of active or passive rest.

Thus, during the work schedule the tested had two rest intervals, the sequence of which was determined by the conditions of the experiment.

As an illustration, we cite in Table 1 the result of observation of G., female.

TABLE 1

Effect of Active and Passive Rest on the Muscular Work Capacity

Date of examination	Examined individual	Age (in years)	Type of rest	Load on left hand	Load on right hand	Work, during the two minutes prior to rest	Work, during the two minutes after first rest	Work, during the two minutes after second rest	Restorative effect of the first rest period	Restorative effect of the second rest period
				in kg		in kg/m			in percentages to the first work	
June 23, 1959	G.	91	Active	2	4	25.36	23.92	15.68	94.3	62
June 24, 1959		91	Passive	–	4	28.53	24.52	21.4	86	75
March 8, 1955	M.	20	Active	4	6	42.34	39.71	32.33	93.8	76.3
March 16, 1955		20	Passive	–	6	37.06	30.22	22.8	81.5	61.5

As seen in Table 1, active rest after the first two minute work produces a more pronounced restorative effect than passive rest, but when it follows the second workout its restorative role is considerably inferior to that of passive rest.

Thus, a slight increase of fatigue in the aged from additional two minute work led to the loss of the positive effect of active rest. For comparison, in Table 1 the data cited are obtained in a young man whose active rest, following the second work period, continues to produce an effective restoration of work capacity as compared to the passive rest period.

It is worth noting that in four out of 28 tested individuals the repeated work was more effective after an active as well as a passive rest period. This fact cannot be connected with the past occupational characteristics of the individuals. However, in these instances the general possible work capacity as determined by their work up to intense fatigue, was smaller than in other tested individuals. This fact permits us to assume that in this case we are dealing with a stable character of the stimulation process in the nervous centers, and with reduced inhibition. The work, therefore, is performed at a high stimulation level but the total work capacity is reduced due to the weakness of inhibition and its effect on the restoration processes.

Consequently, in the evaluation of work capacity in the aged it is important to consider two factors: the intensity of the current work and the total possible work capacity.

In the next group of experiments the load during active rest was raised to 75-80% on the right hand. This load-increase produced in all tests a lower work capacity after an active rest than after a passive rest period. Four individuals, of the age up to 70 years, were an exception.

In the test series with large loads, in nearly all cases, with the exception of one instance, active rest as a rule produced a considerably smaller restoration effect than passive rest (Table 2).

Table 2 shows results of observations on S. Following a two-minute workout with a large load, the active rest (load was equal to only 50% of the load on the right hand), in contrast to two minute work with medium load, loses its advantage over the passive rest, whereas in young people even under these weight categories active rest

was almost always more effective than the passive one. This is attested by the result of examination of K. cited in Table 2. Following two minute work with eight kg, active rest, carried out with 10 kg, proves to be much more effective than passive rest.

Hence, on the basis of the above-cited test-results in which the load was changed on the working group of muscles, as well as during the active rest period, one can arrive at the conclusion that active rest in the aged leads to a positive effect only within certain very narrow limits of optimal loads, i.e., with age, the range of the positive effect of active rest is markedly reduced.

TABLE 2

Effect of Active and Passive Rest on the Muscular Work Capacity

Date of examination	Name of examined	Age (in years)	Type of rest	Load on left hand (in kg)	Load on right hand (in kg)	Work during the two minute period prior to rest (in kg/m)	Work during the two minute period after rest (in kg/m)	Restorative effect of rest (in percentage to the first work)
July 9, 1959	S.	81	Active	3	6	20.58	9.0	43.7
July 1, 1959		81	Passive.....	–	6	32.16	15.42	48
April 6, 1955	K.	19	Active	10	8	38.39	31.38	81.7
April 8, 1955		19	Passive.....	–	8	33.65	20.23	61.1

We mentioned earlier that the majority of authors connect the effect of active rest with the fact that an inhibition process develops during this period in the centers of the opposite group of muscles. This inhibition stimulates the restoration process with the result that an increased work capacity is created. The fact of reduction of the positive effect of active rest in aged, established in our tests, can be explained by the weakening of the inhibition effect on the restoration process at this stage of life.

It is worth noting that repeated exercises led to a well expressed training of muscular work capacity. This was particularly noticeable in the tested individuals who have been regularly attending the tests.

Thus, for example, in G. (age 91) on the 7th exercise the work capacity rose 2-3 fold, as compared to the initial figure. Of particular importance is the fact that during the training period the restorative role of active and passive rest increases. This high rate of increased work capacity, in the course of repeated exercises and training, attests to the considerable potential possibilities of increasing work capacity in advanced and old age.

The obtained facts may serve as a basis for the extensive use of measured physical exercises in this age period.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

SECRETION OF THE EPITHELIUM OF RENAL COLLECTING TUBULES UNDER THE EFFECT OF THE ANTIDIURETIC HORMONE

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(Presented by Active Member AMN SSSR V. M. Karasik)
Translated from Byulleten' Éksperimental' noi Biologii i Meditsiny, Vol. 51, No. 5,
pp. 7-10,
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The mechanism of action of the antidiuretic hormone, according to present-day concepts, is based on the fact that under the effect of this hormone the epithelium of collecting tubules begins to secrete according to the apocrine type. Apparently, the enzyme hyaluronidase enters the composition of this secretion. This enzyme depolymerizes the hyaluronic structures of the interstitium and the intercellular cementing tissue, with the result that the wall of collecting tubules becomes permeable to water [3, 4, 6, 7].

The phenomenon of apocrine renal secretion has been described a number of times in various morphological and electron-microscope works [15, 16], but only A. G. Ginetsinskiy and his co-workers [3, 4] established the connection of this phenomenon with the action of the antidiuretic hormone.

We thought it interesting to investigate the effect of the antidiuretic hormone on the content and distribution of the nucleic acids (RNA and DNA) in the epithelium of the collecting tubules, since it is known that nucleic acids participate in protein synthesis, including the synthesis connected with the process of secretion [1, 2, 5, 8, 9, 10, 12, 13, 14].

METHOD OF EXPERIMENTS

The study was carried out on 16 adult white rats, weighing from 200 to 250 gm, and on 32 ratlings, one to 33 days old which were in a state of hydration and antidiuresis. The kidneys were obtained for examination within an hour after the introduction of water into the animals' stomach in a ratio of 5 ml per 100 gm body weight, or within 30 minutes after a subcutaneous injection of 15-30 m. u. of pituitrin. The material was fixated in Carnoi fluid. The processing for RNA determination was carried out by means of methylgreen-pyronine according to Brachet, or with hallocyanine as per Einarson [11] with an obligatory control by means of ribonuclease. DNA was determined by the Felgen method [11].

In a number of cases, for the histochemical characterization of the secretion, we carried out the reaction on protein as per Daniel-Pierce-Barston [11] which enabled us to elicit simultaneously certain amino acids (tryptophan, tyrosine, and histidine) which enter into the molecular composition of all proteins investigated up to the present time.

RESULTS OF EXPERIMENTS

In hydrated adult animals the collecting tubules are lined with cells of cuboidal epithelium with a centrally located nucleus.

The height of cells is almost uniform, with the result that the lumen of the tubule possesses clearly defined and even outlines. RNA in the cells is found in very small quantities and is evenly distributed over the entire cytoplasm (Fig. 1, a). Thanks to the fine dispersion characteristics of RNA particles an impression is created of a diffuse coloration of the cells' cytoplasm. The RNA content of the epithelium of the collecting tubules is uniform over the entire length of the tubules. Results obtained in eliciting RNA particles as per Brachet and Einarson are completely identical. Upon treatment of the sections with ribonuclease, it is possible to completely split the RNA in all segments of the collecting tubules, excepting the top of the papilla where a very slight coloration of cells' cytoplasm is partially preserved, due to the apparently non-specific character of the cells.

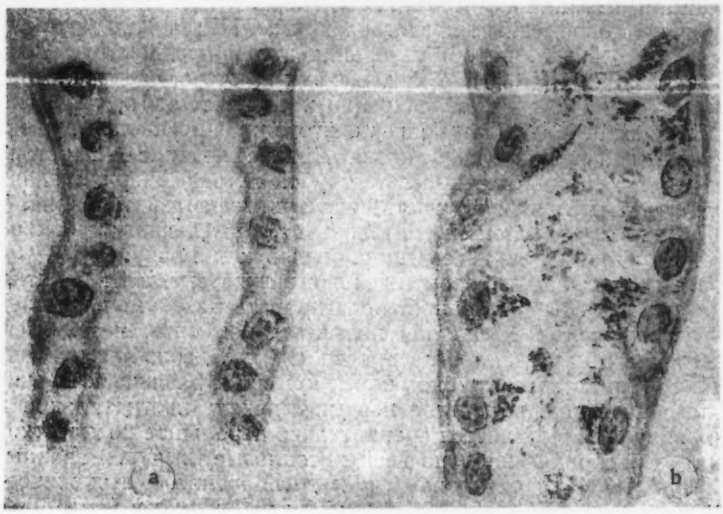

Fig. 1. RNA distribution in the epithelium of collecting tubules of adult rats. a) Under conditions of aqueous diuresis; b) after pituitrin injection. RNA elicited by the Brachet method. Magnification: oc. 10×, obj. 60×, immersion. Drawing made by means of a drawing apparatus.

Under the effect of pituitrin, in the majority of epithelial cells of the initial segments of the collecting tubules situated in the medullar rays, a definite apocrine secretion takes place. The apical cellular ends have indistinct, somewhat loose contours. In some areas a separation of the entire apical pole of the cell into the tubular lumen can be seen. In a number of secretory cells, in their apical part, one can observe formation of fairly large, compactly situated RNA granules. It can also be seen that, in apocrinia, together with the separation of the apical part of cells, there is also a secretion of colored granules in the lumen of the tubule, later elicited in the composition of the secretion which fills the collecting tubules (Fig. 1, b).

Within 30 minutes after the injection of pituitrin, no secreting cells can be found in the epithelium of the tubules at the base and medium third of the papilla, although their lumen is filled with secretion. The epithelial cells in this section are flattened, their nuclei oriented along the tubule and are separated from the lumen by a narrow, thin plasmatic rim. The RNA content in the cytoplasm of these cells is considerably higher than in the corresponding segments of the tubules of hydrated animals. At the apex of the papilla the character of epithelial lining and the RNA quantity in the cellular cytoplasm is not altered.

In contrast to what can be observed in the RNA study, the change in the content and distribution of DNA, determined according to Felgen in the membrane and chromatin of the epithelial nuclei of the collecting tubules following injection of pituitrin, could not be ascertained.

According to modern concepts, RNA serves as a matrix for the synthetized protein, and its formation is always preceded by an increased RNA concentration in the cells. On this basis, we would expect the formation of a protein secretion also under apocrine secretion. Upon verification of this assumption via histochemical

reaction on protein as per Daniel–Pierce–Barston, it was found that the secretion obtained in the tubular lumen had a large quantity of amino acids of histidine, tryptophan, and tyrosine. Thus, the secretion of the epithelium of collecting tubules, under antidiuretic conditions, is undoubtedly of protein nature.

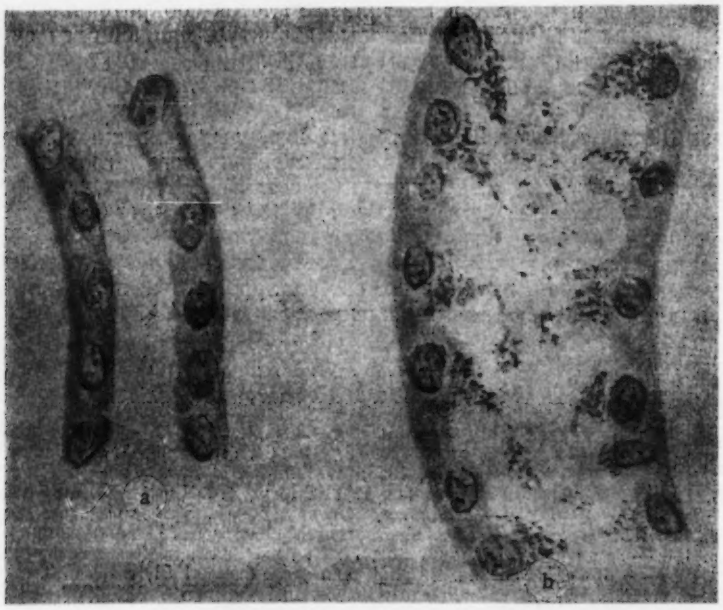

Fig. 2. Development of renal reaction to the antidiuretic hormone during ontogenesis. a) Absence of a reaction to the injection of pituitrin in the collecting renal tubules of a 13-day-old ratling; b) apocrine secretion of the epithelium of collecting renal tubules of a 31-day-old ratling after the hormone injection. RNA elicited by Brachet method. Magnif.: oc. 10×, obj. 60×, immersion. Drawing made by means of drawing apparatus.

It is known that kidneys of newborn are insensitive to the antidiuretic hormone, and that the property of reacting to the hormone by means of a canalicular antidiuresis originates only during the postnatal ontogenesis. In this connection, we studied the distribution and content of nucleic acids in the renal collecting tubules at various periods of postnatal development. During the first three weeks of postnatal life a small quantity of RNA, evenly distributed over the cellular cytoplasm, is elicited in the epithelium of the tubules. No difference is yet observable during this period in the RNA content in hydrated and dehydrated animals. Neither are there any changes noted after the injection of pituitrin (Fig. 2, a). Only starting on the 22nd day, the hormone injection induces an enhanced RNA synthesis in the epithelium of the collecting tubules.

During the same period of time characteristic pictures of apocrine secretion make their appearance.

Toward the 31st-33rd day these changes are as clearly expressed as in adult animals (Fig. 2, b). The coincidence should be noted of the periods of appearance of RNA changes in the cellular cytoplasm of the collecting tubules and the formation of renal hyaluronic structures which represent the action substrate of the antidiuretic hormone [6].

In summarizing all above-stated, we can arrive at the following conclusion.

In the epithelium of the collecting tubules of adult animals and ratlings over 20 days old, under the effect of the antidiuretic hormone a redistribution and enhanced synthesis of RNA takes place; this process precedes the formation of the secretion discharged into the lumen of the tubule. Histochemical analysis of the secretion confirmed its protein nature.

Thus, the idea that the secretion, discharged by the epithelium of the collecting tubules, contains a hyaluronidase enzyme finds its new, indirect corroboration.

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THE EFFECT OF THE SOMATOTROPIC HORMONE AND CORTISONE ON THE LIVER INSULINASE ACTIVITY IN INFANTILE AND SEXUALLY MATURE RATS

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(Presented by Active Member AMN SSSR V. G. Baranov)
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In our previous work [2] we demonstrated that the activity of liver insulinase in infantile rats (21-30 days old, weighing 30-50 gm) is reduced in comparison to the insulinase activity of sexually mature rats (1-2 years old, weighing over 170 gm). Administration of chorionic gonadotropine (25 mg twice daily) to sexually immature male and female rats, of estradiole-dipropionate (100 m. u.) to female and testosterone-propionate (0.4 mg) to male for a period of five days increases the activity of their liver insulinase, whereas administration of testosterone-propionate (two mg daily, for five days) to sexually mature rats does not affect the activity of liver insulinase. These data attest to the fact that sexual hormones do not directly activate insulinase, but merely contribute to the formation of this function during ontogenesis.

In view of the fact that the insulin activating system of the liver (insulinase) represents one of the factors of the extra-pancreatic inactivation of insulin, and that the enhancement of its activity may play a definite part in the pathogenesis of certain forms of diabetes mellitus, it seemed to us essential to elucidate the manner in which other hormones affect this system, in the first place, those which may contribute to the development of diabetes mellitus. A solution of this problem would enable us to elicit the role of the insulin activating system of the liver within the mechanism of the diabetogenic action of these hormones.

In the present work we set ourselves the task of investigating the effect of the somatotropic hormone of the hypophysis and cortisone, which as is known may play a definite part in the pathogenesis of certain forms of diabetes mellitus [3], on the activity of the liver insulinase of sexually mature and infantile rats.

METHOD OF EXPERIMENTS

Experiments were staged on infantile rats (aged 21-30 days, weighing 30-50 gm) and sexually mature rats (aged one to two years, weight 190-270 gm). The somatotropic hormone* was administered for five days, twice daily to infantile rats at 0.5 mg and to sexually mature rats at 1.5 mg. Cortisone was administered to infantile and mature rats at 1.0 mg per 100 gm body weight daily, for five days. Determination of insulinase activity was carried out by Mirskii method [4] with I^{131} labeled insulin as follows: the rats were decapitated, their liver removed and immediately homogenized on cold with a quadruple volume of 0.067 M phosphate buffer. The homogenate was centrifuged for 20 minutes in a refrigeration centrifuge at 1-5° and 10,000 revolutions per minute.

* In our work we used the somatotropic hormone obtained in a manufacturing laboratory and tested by means of the tibial test at the Department of Biology of the All-Union Institute of Experimental Endocrinology.

After centrifugation, the fluid over the precipitate was filtered off through gauze. One ml of this fluid and one ml of 0.067 M phosphate buffer with pH 7.5 containing 0.25 mg of I^{131} labeled insulin were put into the centrifuge test tube. One ml of this mixture was placed on the target for the determination of total radioactivity, and 0.2 ml into a centrifuge test tube containing 0.8 ml of 0.067 M phosphate buffer for determination of the free radioactivity of insulin with I^{131} not incorporated in the molecule. For this purpose, several ml of soluble protein and one ml of a 10% solution of trichloracetic acid were added so as to precipitate the insulin bound I^{131}. The mixture was centrifuged, the fluid over the precipitate poured off into a centrifuge tube, and 0.2 ml from this tube were placed on the target for the determination of radioactivity of the non-precipitated I^{131}. After determining the total and free radioactivity, the mixture was kept for 30 minutes in a thermostat at 37° and the free radioactivity was again determined. The insulinase activity was expressed in percentages of I^{131} which had been split off, as the result of enzymic fission of insulin; the latter was determined according to the extent of the increase of free I^{131} radioactivity during the 30 minutes incubation period. All calculations were made on one ml of incubation mixture. In order to determine the possible spontaneous splitting off of I^{131} we conducted simultaneously a control test in which the homogenate was substituted with one ml of 0.067 M phosphate buffer.

RESULTS OF EXPERIMENTS

As seen in Fig. 1, a 5-day administration of the somatotropic hormone to sexually mature rats causes an increase of liver insulinase activity in them. Whereas in the control group the insulinase activity prior to I^{131} splitting off equalled 26.1% on the average, in the tested group it proved to be 34.4%, i.e., increased by 20.3%. Administration of the somatotropic hormone to infantile rats revealed a more considerable increase of insulinase activity. Whereas in the control group the insulinase activity in infantile rats was 14.6% after the I^{131} split off, in the tested group after a five day administration of the somatotropic hormone it was 32.4%, i.e., two-fold higher, than the activity in the control group.

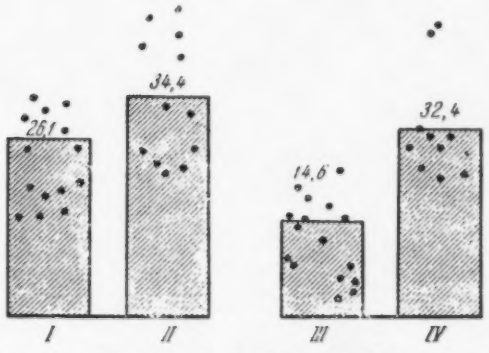

Fig. 1. Effect of administration of the somatotropic hormone on the activity of the liver insulinase of mature and infantile rats. Sexually mature rats: I) Norm; II) subcutaneous injection of the somatotropic hormone, 1.5 mg daily for five days; infantile rats: III) norm; IV) subcutaneous injection of the somatotropic hormone, 0.5 mg daily for five days.

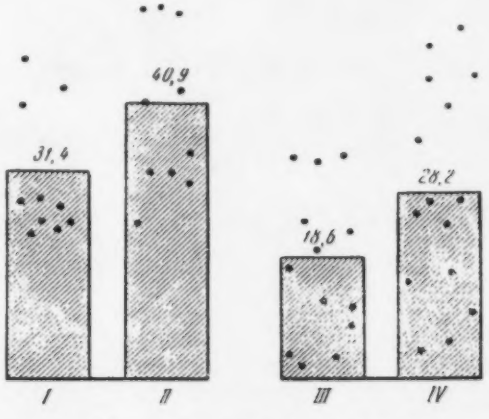

Fig. 2. Effect of cortisone administration on liver insulinase activity in sexually mature and infantile rats. Mature rats: I) Norm; II) administration of one mg/100 gm of cortisone, daily for five days; infantile rats: III) norm; IV) administration of one mg/100 gm cortisone, daily for five days.

We demonstrated in our previous work [1]that antidiabetic sulfonamide preparations (nadisan) reduce the activity of liver insulinase. Recently, Mirskii and his co-workers [5] have found that administration of tolbutamide to dogs protects them from developing diabetes resulting from the injection of the somatotropic hormone. This is, presumably, connected with the inhibiting effect of tolbutamide on the insulinase activity of the liver.

An action analogous to somatotropic hormone, i.e., an increase of insulinase activity, was elicited also upon injection of cortisone to sexually mature and infantile rats (Fig. 2). In mature rats, the insulinase activity in the control group comprised 31.4%, in the tested group—40.9%, or an increase of 30.2%. In infantile rats, the

indices were 18.6% and 28.2%, respectively, i.e., under the effect of cortisone the insulinase activity increased over 1½ times.

Thus, administration of the somatotropic hormone and cortisone increases liver insulinase activity in mature and infantile rats, and it is somewhat more pronounced in the latter. The obtained data permit us to assume that in the mechanism of the diabetogenic effect of these hormones a definite role may be played by their stimulating effect on insulinase activity.

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PARTICIPATION OF THE VASCULAR COMPONENT IN THE HORMONAL MECHANISM OF PANCREAS STIMULATION

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With the discovery by Beilis and Starling [9] of the hormonal mechanism of pancreas stimulation, the problem of the physiological action of secretin has been discussed many times under its most diverse aspects [1]. Particular attention of the investigators was attracted to the problem of direct and indirect effect of the hormone on the secretory cells of the pancreas. A theory has been advanced that the secretory effect obtained via administration of an HCl solution into the duodenal cavity and injection of the extracts of the duodenal mucosa was caused by an increased blood supply to the pancreas and the effect of vasodilating substances.

However, experiments with the use of secretin, purified of vasodilators, as well as crystalline secretin, showed that this hormone stimulates pancreatic activity without increasing its blood flow [13]. The latter phenomenon takes place only in cases when an obstacle to blood outflow is created which leads to an increased secretory pressure in the duct [10, 11, 12].

Thus, the majority of authors accept at present the specificity of secretin action and deny the participation of the vascular factor in this process. However, such an assertion is based on the results of acute experiments, when in an animal which had been anesthetized and subjected to surgical trauma, the secretion of pancreatic juice and the blood flow rate in pancreatic vessels were simultaneously recorded. It is possible that this form of an experiment is acceptable for the solution of this problem, and it is, therefore, possible to be in accord with the concept of the specificity of secretin. But, when we speak of the dynamics of pancreatic secretion under normal physiological conditions, it is scarcely possible to concur with the categorical denial of the role of the vascular component in this process.

This assumption follows not only from the general physiological regularity, according to which each functioning organ is better supplied with blood, but from facts obtained in recent years in regard to the blood supply of the stomach during its secretory activity [2, 3]. In chronic experiments on dogs with isolated ventricles, as per Pavlov, and special electrodes leading from thermoelectric Rein clocks and implanted in a gastric vessel, it was established that the food intake causes long before the appearance of secretion an accelerated blood flow in gastric vessels which increases with the development of the secretory process, reaches its maximum during the period of the most intensive function of the gastric glands and abates with the cessation of secretion. Consequently, these experiments demonstrated the participation of the vascular component not only in the complex reflex phase, but also in the neuro-chemical secretory phase when the glandular activity is caused mainly by hormonal influences.

This fact prompted us to carry out an experimental study of the vascular component in the hormonal mechanism of pancreatic stimulation.

METHOD OF EXPERIMENTS

The study of pancreatic secretion and the blood flow rate in the gland was carried out on dogs, weighing 15-20 kg, in acute and chronic experiments.

The acute experiments were conducted under urethan anesthesia (10% solution intravenously). The secretion was collected in a cannula inserted in the large pancreatic duct; the small duct was ligated; the stomach was separated from the duodenum at the expense of the mucous membrane. Recording of the secretion was taken every minute via pneumatic transmission to a horizontally situated menometric scale; in addition, the total quantity of secretion, collected during the entire secretory period, was measured in milliliters. An interval of not less than an hour was maintained between the employment of each stimulus. Changes in the blood flow rate were determined in Arteria pancreaticoduodenalis by means of a Rein thermoelectric clock. These changes were elicited by processing the photokymograms and were conditionally expressed in millimeters of the rise or fall of

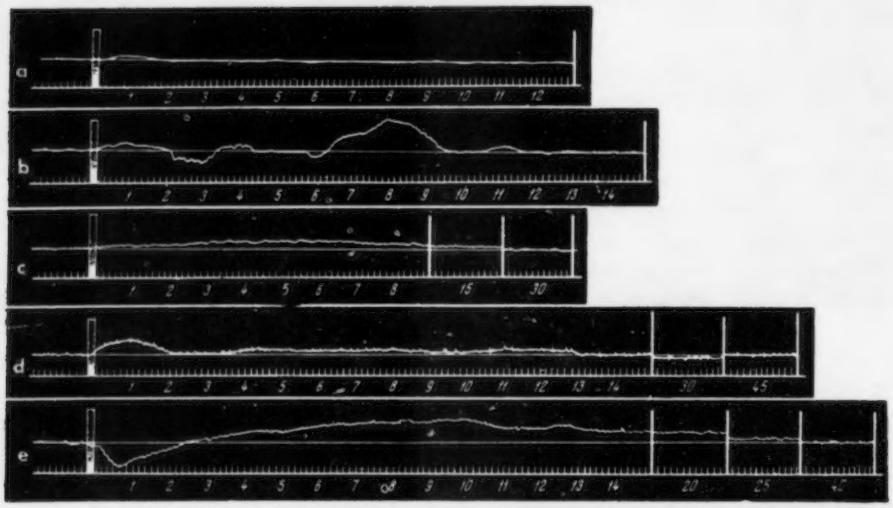

Fig. 1. Dynamics of changes in the blood flow rate in the pancreas upon administration of secretin. Designation of curves (top to bottom): photokymogram of the blood flow rate; mean initial level of blood flow (horizontal lines); time mark in seconds (each division – 10 seconds; minutes are designated with numbers). a) Administration of "Mebiol-Secretin" in acute experiment; b) administration of the extract of duodenal mucosa in acute experiment; c) administration of "Lilly-Secretin" in chronic experiment; d) administration of "Mebiol-Secretin" in a chronic experiment; e) administration of the extract of duodenal mucosa in a chronic experiment. Columns on the left – time of administration of secretin or extract.

the blood flow in relation to its initial level. As a stimulus of pancreatic secretion, we employed 10% solutions of crystalline "Lilly-Secretin" and "Mebiol-Secretin" in 1-2 ml quantities and an extract from cat's duodenal mucosa obtained by the usual method in 10-20 ml quantities. These substances were administered intravenously. As control, we employed a physiological solution injected in the same quantities. A total of 32 acute experiments was staged; the secretory effect of secretin was tested 65 times.

Chronic tests were carried out on four dogs with a chronic fistula of the pancreatic duct, as per Pavlov, (two dogs), and with electrodes implanted into the pancreatic artery, from Rein's thermoelectric clocks. A total of 39 chronic experiments was carried out.

The obtained secretion was examined for the content of total and free lipase, according to the Bondi-Rozhkova method, trypsin – per Serensen, and amylase – per Volgemuth. The titrated alkalinity and viscosity of the secretion were also determined.

RESULTS OF EXPERIMENTS

The results of acute experiments were as follows. Administration of extracts from duodenal mucosa of secretin solutions produced non-uniform vascular effects. Most frequently, a very slight and transitory acceleration of the blood flow was observed (Fig. 1, a); this effect was noted in 44 cases out of 65 (68%). A definite increase of the blood flow was observed in five cases only (7%); in the rest, an increase of blood flow alternated with its reduction (Fig. 1, b). This took place in 25% of cases; it usually originated when extracts had been used.

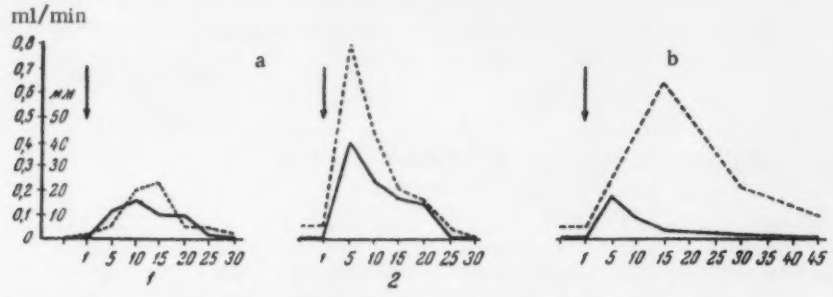

Fig. 2. Correlation between the vascular and secretory reactions of the pancreas following administration of secretin. —— change of blood flow rate (in millimeters); ---- change of intensity of pancreatic secretion (in milliliters per minute). Along the ordinate axis: quantity of secretion (in milliliters), blood flow rate (in millimeters); along the abscissa axis: time (in minutes). 1) Experiment January 13, 1960; 2) experiment January 20, 1960; a) Administration of extract of duodenal mucosa; b) administration of "Mebiol-Secretin." Arrows indicate the moment of secretin administration.

Secretin solutions most frequently caused an abundant pancreatic secretion. The latent period of secretion did not exceed 55-70 seconds. Maximal secretion was observed on the 2nd-3rd minute. The duration of the effect was 15-20 minutes. Total amount of secretion fluctuated in various dogs within wide limits (from 5 to 18 ml).

In some experiments a definite connection was elicited between the secretory activity of the pancreas and the character of changes in the blood flow rate. Thus, for instance, in the test with double administration of one ml of a 10% solution of "Mebiol-Secretin" the blood flow acceleration after the first administration of the solution took place within 200 seconds, after the second administration—within 20 seconds. Correspondingly, the volume of secretion for the entire secretory period comprised 10.5 ml after the first administration of the solution of secretin, and 15 ml—after the second administration. The enzymic content in the secretion in cases of a rapid and intensive acceleration of the blood flow and increased secretion was usually lower, as compared to cases when the acceleration of the blood flow proceeded slowly after a prolonged latent period and a scanty secretion.

However, such clear dependence between changes of blood flow rate and the intensity of pancreatic performance did not take place in every case—a fact which in our opinion can be connected with the conditions of an acute experiment and with the difference in the depth of narcosis in particular.

We obtained clearer results in chronic experiments. An important feature of these results, as compared to the results of acute experiments, was the blood flow acceleration in response to secretin administration. It was observed in all four dogs. The range of blood flow acceleration in various dogs at diverse time periods fluctuated between 7 and 40 mm.

The effect of "Lilly-Secretin" was studied on dogs Dik and Kozyr'. The latent period of the vascular reaction is generally 10-25 seconds, the duration of the reaction comprised 15 minutes in Kozyr' and 30 minutes in Dik. The blood flow reached the highest acceleration during the first 6-8 minutes (Fig. 1, c).

In the dog Ramzai with a permanent fistula of the pancreatic duct, the increase of blood flow rate in the pancreatic artery upon administration of "Mebiol-Secretin" took place within 5-10 seconds after reaching its

maximum (18-20 mm) which occurred during the first few minutes after the injection of secretin; within 15-20 minutes, the blood flow rate reverted to the initial level (Fig. 1, d). The pancreatic secretion in these cases would start only within 50-60 seconds after injection of secretin and lasted 20-30 minutes; the maximal secretory activity was usually reached toward the 15th minute. During the entire secretory period about 15-20 ml of juice was secreted, on the average (Fig. 2, b).

In the dog Tsygan, also with a permanent fistula of the pancreatic duct, the secretory and vascular reactions to intravenous administration of the extract from the duodenal mucosa were investigated. Upon its injection in the blood, the blood flow acceleration (10-40 mm) occurred at various periods—from scores of seconds to several minutes—and lasted 30 to 40 minutes (Fig. 1, e). The highest rise occurred on the 10th-12th minute. Pancreatic secretion started after the emergence of vascular reaction and reached its maximum on the 5th-15th minute; it ceased within 30 minutes after the administration of the extract (Fig. 2, a).

Correlation of the Time Between the Appearance of Secretion and the Change of the Blood Flow Rate in the Dog's Pancreas After the Intravenous Administration of Secretin

Date of experiment	Time of appearance of secretion and blood flow change (in seconds)		Date of experiment	Time of appearance of secretion and blood flow change (in seconds)	
	Blood flow	Secretion		Blood flow	Secretion
January 9th	120	210	January 16th	120	380
January 11th	20	50	January 17th	80	200
January 13th	125	240	January 18th	10	40
January 14th	170	360	January 19th	360	50
January 15th	130	360	January 20th	20	60

There was usually a correspondence between the secretory and vascular reactions, in the sense that a higher acceleration of the blood flow was accompanied by a more intensive pancreatic secretion and the changes in the blood flow rate always preceded the emergence of the secretory effect (see table).

Most frequently a direct interdependence was observed between the increased rate of the blood flow and the enzymic content of the juice which had been secreted in response to secretin administration: with the increase of blood flow rate, a larger quantity of enzymes was secreted. Analogous relationship was noted between the magnitude of the blood flow rise and the titrated alkalinity of the secretion. Pancreatic juice, secreted in response to secretin administration, always had lower viscosity than the juice secreted in response to food stimulation.

Thus, the obtained results attest to the fact that extracts from the duodenal mucosa as well as pure secretin not only stimulate the secretory pancreatic activity, but also increase its blood supply. Since this effect is less pronounced under conditions of an acute experiment and is always present under normal physiological conditions, there is reason to believe in the importance of the development of this effect in the normal functioning of the nervous system, especially after investigations of a number of authors [4-8] have established the dependence of the secretin stimulation of the pancreas on the nervous system.

Changes in the pancreatic blood supply appear before the start of secretion. The marked constancy of these reactions, observed in chronic experiments, permits the assumption that such changes of the blood flow rate in the organ are very essential to the course of normal pancreatic secretion. At the same time, our data indicate the principal possibility of a humoral stimulation of the pancreas without changes in its blood supply. However, since these phenomena had been elicited only under conditions of an acute experiment with the use of narcosis and manipulations of a traumatic character to the nervous system, they can scarcely be regarded as physiological reactions.

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CHANGES IN THE ACTIVITY OF THE CARDIOVASCULAR SYSTEM AND RESPIRATION IN DECORTICATION

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pp. 18-22,
Original article submitted May 20, 1960

The problem of the effect of decortication on the activity of the cardiovascular and respiratory systems has been the subject of investigation by the laboratories of B. I. Bayandurov [7], V. N. Chernigovskii [28], E. A. Asratyan [1-4], S. I. Frankshtein [25, 26], M. V. Sergievskii [21, 22], and N. Yu. Belenkov [8], as well as the works of N. T. Shutova [29], F. Z. Meerson [17, 18], A. A. Uzbekov [24], P. F. Avtonomov, A. A. Kremin [2], and other authors. There are available data that the disturbance of the anatomical unity of the higher sections of the central nervous system leads not only to functional, but also to structural changes of the elements of the cardiovascular system [11, 27]. This problem has not been sufficiently studied as yet, especially under conditions of a chronic experiment, and the method of electrocardiographic examination was employed in a few works only [14, 18]. Also insufficient studies have been devoted to the reactivity of the cardiovascular system and respiration of decorticated animals subjected to specific strains which contribute to the elicitation of changes in their functional state.

Our previous experiments demonstrated [6] that functional disturbances of the circulation and respiration apparatus in decortication are not always clearly manifested. In this connection, the task of present investigations was elucidation of the range of compensatory possibilities and the study of the cardiovascular system and respiration after a uni- and bi-lateral removal of the cortex of the large cerebral hemispheres. We used for this purpose pituitrin—the hormone of the posterior hypophyseal lobe which possesses strong vasomotor properties.

METHODS OF EXPERIMENTS

The tests were carried out on rabbits of the chinchilla species, weighing 2.0-2.5 kg. The blood pressure in these animals was measured via the blood stream prior to the operation and at various periods after it. The blood pressure and respiration were recorded on a kymograph. The electrocardiogram (ECG) was recorded in three standard leads—I, II, and III—by means of a battery powered ÉKP-4 electrocardiograph, and in a number of tests with a one-channel mesh ÉKPS-1 electrocardiograph; simultaneously, for visual observation, an electrocardioscope was cut in. At the start of the experiment, the initial level of blood pressure and respiration was established and an ECG taken. Then, the rabbit received intravenously pituitrin, 0.3-0.6 units per kg weight. The blood pressure, respiration, and ECG were recorded at the moment of pituitrin administration and within 30 seconds, 1, 3, 5, 10, 20, and 30 minutes after injection of the hormone. Nembutol narcosis was employed (0.025-0.03 gm per kg weight). Decortication and feeding of decorticated animals was carried out, according to the previously described method [5]. A total of four series of experiments on 45 animals was carried out.

The first three series were conducted on 12 rabbits which were examined within 1, 2, and 4 weeks following decortication; the 4th series—on nine normal rabbits in which the changes of the ECG, blood pressure, and respiration under the effect of pituitrin were studied under conditions of acute experiments. In the experimental animals, a uni- and bi-lateral decortication was performed; thus, the first three series comprise equal numbers of rabbits with uni- and bi-lateral decortication.

RESULTS OF EXPERIMENTS

Following decortication, especially a bilateral one, the postoperative period had a difficult course. But the basic indexes of the cardiovascular activity (blood pressure, rate of cardiac contractions, ECG, and respiration) showed only slight changes following decortication. During the first few days after the operation, a slight retardation of the rhythm of respiratory movements was noted, and in a number of instances a slight increase of their amplitude. More marked and prolonged respiratory changes were noted in bilateral decortication (Fig. 1).

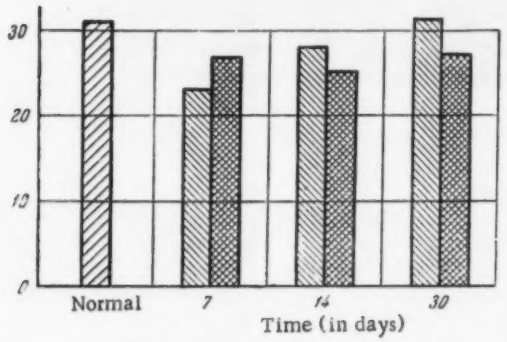

Fig. 1. Changes in the rate of respiratory movements in a rabbit after decortication. Obliquely crosshatched columns—unilateral decortication, mesh-columns—bilateral decortication.

During this period there were changes observed in the rate of cardiac contraction. A certain deceleration of the cardiac rhythm was observed in all experimental animals directly after the operation. However, already within a week after decortication the pulse rate in some animals reverted to norm. These findings are possibly explained by the sharp reduction in the activity of the animals. Various external stimuli are unable to exert their effect when the cortical apparatus which regulates organic functions is absent.

Injection of pituitrin caused (in non-operated rabbits) either a reduction of the amplitude of respiratory movements, or more often their complete cessation. Normalization took place within 10-15 minutes. Restoration of respiration following administration of pituitrin in decorticated animals proceeded slower than in norm. Normalization of respiration in rabbits within one and two weeks after decortication took place within 30-40 minutes; in animals, examined a month after decortication, the character of restoration of respiration after pituitrin injection differed little from that of the control animals.

Injection of pituitrin in control animals caused a rise in the level of arterial pressure of 20-40 mm of mercury column, reaching its maximum on the 2nd-3rd minute after the injection. The maximum rise of arterial

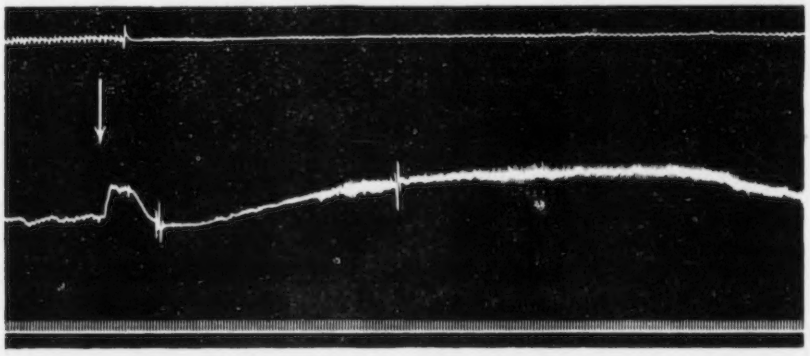

Fig. 2. Changes in arterial pressure and respiration under the effect of pituitrin in a control rabbit. Designation of curves (top to bottom): arterial pressure; time mark (one second). Arrow points to the moment of pituitrin injection.

pressure was accompanied by a sharp increase of the amplitude of cardiac contractions. Normalization of these indexes took place within 10-15 minutes after the injection (Fig. 2). The electrocardiographic changes in intact rabbits under the effect of pituitrin consisted of the development of bradycardia, prolongation of the diastole, increase of R and T waves, and reduction of the P wave. These phenomena increase for a period of four minutes, then the ECG reverts to norm toward the 10th minute.

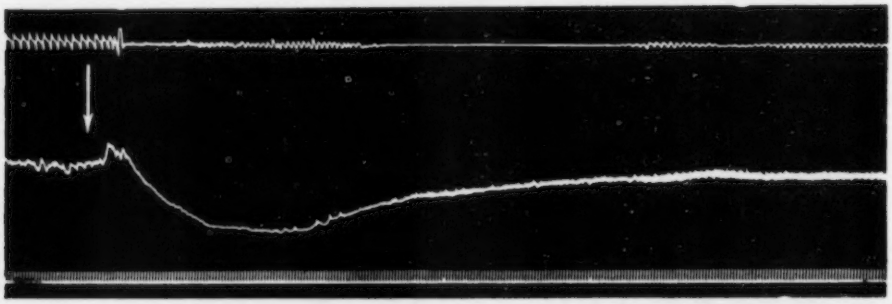

Fig. 3. Changes in arterial pressure and respiration under the effect of pituitrin in a rabbit after a bilateral decortization. Designations the same as in Fig. 2.

Within a week after decortication, there was observed under the effect of pituitrin a more pronounced deceleration of the cardiac rhythm than in the control animals and the emergence of occasional ventricular extrasystoles. Normalization of ECG indexes is retarded by 20-25 minutes. The pressor effect from the injection of pituitrin to these animals is less manifested than in control animals. Following normalization of cardiac activity, the arterial pressure was still at a lower level than prior to pituitrin administration.

Two weeks after decortication, injection of pituitrin caused more pronounced changes in the activity of the cardiovascular system: immediately after the injection the arterial pressure decreased by 20-40 mm, and the subsequent pressor effect did not exceed 10-15 mm (in norm it usually equals 20-40 mm). The arterial pressure kept decreasing and became stabilized at a lower than initial level. Evidence of reduction of the contractile property of the myocardium as well as deceleration of the rhythm of cardiac activity, following pituitrin administration, can be observed even 20-30 minutes after the injection.

Normalization of ECG in rabbits within four weeks after decortication against the background of the pituitrin action is even more retarded. Injection of pituitrin caused rotation of the cardiac axis to the left (negative T wave), as well as symptoms of overtaxation of the left ventricle. The pressor reaction of the cardiovascular system to pituitrin administration is considerably less expressed as compared to norm and is very often absent altogether (Fig. 3).

Thus, our studies demonstrated that under conditions of decortication there is an inadequacy of compensatory possibilities of the cardiovascular system and respiration which manifests itself, when increased demands are brought upon the organism. It is possible that decortication has impaired the functional capacity of the lower sections of the central nervous system, thus leading to the above-described disturbances.

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EFFECT OF BODY COOLING ON THE MICROPHONE EFFECT OF THE COCHLEA

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According to the data in literature, ice cooling of the cochlea of animals [4, 7] and general cooling of the organism in the presence of subacute ischemic hypoxia [7] produces a distortion of the sinusoidal form of the electric potentials of the cochlea and reduction of their magnitude up to complete disappearance. Warming of the labyrinth increases the intensity of the electric reaction [6] and raises the upper limit of the received sonal frequencies [5].

The aim of our work was to study the effect of general cooling of the organism as well as certain forms of local cooling on the function of the internal ear via determination of the electric reaction (microphone effect) of the cochlea which, as is known, represents one of the indexes of the auditory function of the internal ear.

METHOD OF EXPERIMENTS

Rabbits of the chinchilla species, aged 6 months to two years, were operated upon under barbamyl narcosis in order to approach the cochlear foramen ovale – the point of best manifestation of the electric reaction of the auditory reception apparatus. The functioning parts of the middle ear and labyrinth were left intact. We recorded the electric potentials of the cochlear action which were carried off from the foramen ovale upon stimulation of the auditory apparatus with pure sounds. The sonar vibrations of needed frequency and intensity were generated by an audiometric device consisting of type ZG-10 generator which had been provided with an attenuator, and an electrodynamic reproductor from which they were relayed via sound-transmitter to the animal's ear. The action potentials were carried off by platinum electrodes, one mm in diameter. The active electrode was attached near the oval foramen, and the indifferent, needle-shaped electrode was inserted in the muscle at the edge of the surgical wound. Recording of the electric potentials was made with a cathode ray oscilloscope of the EO-7 type (for visual observation of the electric reactions) and with a simultaneously cut-in oscillograph-spectrometer with a high frequency photokymocamera (for the recording of electric potentials on the cinefilm at the rate of movement equalling 130-160 cm per second).

A total of 25 experiments were conducted on 19 rabbits. In order to ascertain the effect of general cooling of the body on the microphone effect of the cochlea (11 tests on eight rabbits), we determined the range of received sounds, the voltage of the cochlear potentials upon stimulation with tones of above-threshold uniform intensity – 70 decibels (over the 0.000204 bar level), and the liminal magnitudes of the tone intensities which stimulated a minimal electric cochlear reaction at frequencies: 100, 200, 500, 1000, 2000, 3000, 4000, 6000, 8000, 10,000, 12,000, 14,000, and 16,000 oscillations per second. These measurements were taken prior to general cooling, in the state of hypothermia (rectal temperature 25°-26°), as well as (in seven tests) after warming the animals to the initial body temperature. The hypothermia state was obtained by covering the exposed surface of the animal with ice.

In order to elucidate the effect of local cooling on the cochlear microphone phenomenon (14 tests on 11 rabbits) we made similar determinations of the electric reaction as in tests with the study of general hypothermia – during the transitory (for a period of 10 seconds) local cooling, then directly after its cessation, and up to the moment of restoration of the initial temperature of the tissues of external auditory canal, ear drum, and the external wall of the labyrinth. Measurements of the temperature of these tissues were made with an electrothermometer. Cooling was produced via introduction of ethyl chloride or a piece of ice either in the external auditory canal or (for direct cooling of the labyrinth) into the middle ear cavity (bulla tympani) through a trepanation opening in its outside wall. The possibility of a mechanical obstruction to the vibratory movements of the ear drum was excluded because ethyl chloride rapidly evaporated, and the water formed from melting ice was quickly removed prior to recording.

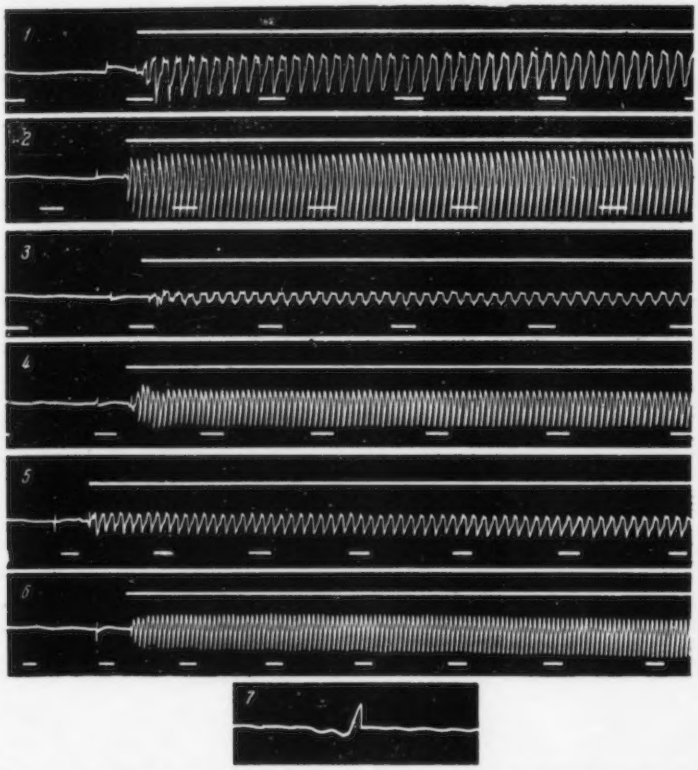

Fig. 1. Oscillograms of electric potentials of the cochlear reaction of a rabbit (experiment No. 8) recorded prior to total cooling of the animal, as well as in the state of hypothermia and after warming. Stimulation of the auditory apparatus with pure sounds, 500 and 1,000 vibrations per second, respectively. 1 and 2) Prior to cooling (rectal temperature 37.5°); 3 and 4) the same, in the state of hypothermia (rectal temperature 25.5°); 5 and 6) the same after restoration of the temperature to the initial level; 7) standard impulse (500 μV). Designation of curves (top to bottom): stimulation mark; time mark (20 msec). To read from left to right.

RESULTS OF EXPERIMENTS

In rabbits subjected to total cooling the range of received sounds which were responsible for the stimulation of the cochlear electric reaction did not change substantially. However, the magnitude of the electric reaction

(voltage of the potentials) of the cochlea, upon its stimulation with sounds of supraliminal intensity, decreased (with some exceptions), the thresholds of origin of this reaction rose, while the sinusoidal form of the electric potentials became distorted. At some frequencies in several tests, the reaction intensity rose or remained unchanged and the thresholds of its origin decreased or showed no change. Quite frequently we were unable to note any correlation between changes in the magnitude of the electric reaction, following stimulation with sounds of supraliminal intensity (70 decibels), and the direction of liminal changes of the origin of this reaction. At times we observed paradoxical relations, where the reaction intensity would decrease, while the thresholds of its origin would decrease somewhat or would not change at all, and sometimes a reverse relationship emerged. The extent of changes of the intensity of the electric reaction and its threshold of origin in various animals at the same degree of hypothermia differed.

An identical reduction of the temperature may exert a different effect on the electric reaction of the cochlea not only in various animals, but even in response to sonar stimuli of varying frequency in the same animal. Apparently, in hypothermia a change takes place in the reflex influences which may exert a varying, "mosaic" effect on diverse cochlear receptor structures which receive various sonar frequencies. This corresponds with our data, obtained earlier [2, 3], on the "mosaic" character of the reflex regulation of the functional state of the sonar receptor apparatus.

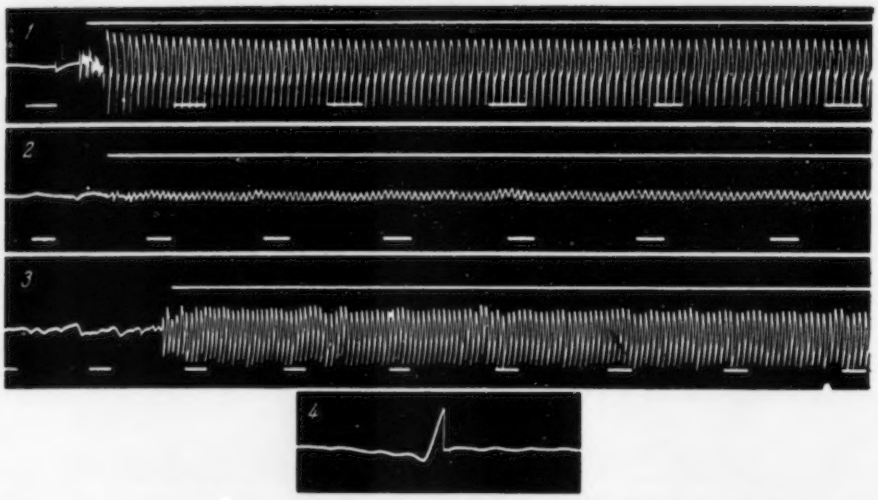

Fig. 2. Oscillograms of electric potentials of the cochlear reaction of a rabbit (experiment No. 19) recorded prior to cooling of the external auditory canal and ear drum, and after their brief (10 seconds) cooling with ice. Upon stimulation of the auditory apparatus with a pure sound of 1,000 vibrations per second [temperature of the external wall of the labyrinth (promontorium) 35°]. 1) Prior to cooling; 2) the same within 10 minutes after cessation of cooling (temperature of the external wall of the labyrinth 34.9°); 3) the same within 3½ hours after cessation of cooling; 4) standard impulse (500 μV). Designations the same as in Fig. 1.

At the moment of restoration of the body temperature (by warming) to its initial level, no complete restoration of intensity of the cochlear electric reaction and the thresholds of its origin would necessarily take place in each case; considerably more frequently the restoration was incomplete (Fig. 1). At times, no restoration took place at all; moreover, upon stimulation with certain frequencies, a further reduction of the reaction intensity and increase of the thresholds of its origin was observed.

Restoration of the reaction intensity, upon stimulation with sounds of supraliminal intensity (70 decibels), often did not correspond to the direction of changes of the thresholds of its origin.

Apparently, in hypothermia fairly stable changes may take place, since at the moment of temperature restoration to the initial level no complete restoration of the functional state of the cochlear receptor structures which generate electric potentials had taken place in the overwhelming majority of cases.

A local brief (10 seconds) cooling of the external auditory canal and ear drum with ethyl chloride or ice rapidly caused a considerable narrowing of the band of sonar receptor frequencies (at the expense of reduction of the upper and increase of the lower limit), as well as a sharp reduction in the intensity of the cochlear electric reaction at all average frequencies. In case of average frequencies, the disappearance of the reaction could be observed only upon more prolonged cooling.

After the restoration of local cooling, the cochlear electric reaction again began to increase as manifested in the form of a gradual widening of the band of sonar frequencies which started the reaction (at the expense of the successive rise of the upper and lower threshold), and the voltage increase of the action electro-potentials (Fig. 2). A full restoration of the reaction (to the initial level) took place within 3½-4 hours after the cessation of local cooling.

Upon a direct and brief cooling of the labyrinth via introduction of ethyl chloride into bulla tympani, the cochlear electric reaction changed to the same extent as upon cooling of the external auditory canal and ear drum, but it was more rapid.

It was found that in local brief cooling the electric reaction changes had not been caused by the reduction of the temperature of the cooled tissues, as attested by the results of measurements of their temperature immediately after the removal of the cooling substance. Thus, in nine tests out of 14 the temperature of tissues showed no change, and in the rest it decreased only by a fraction of a degree and was restored within a few minutes after the removal of the cooling substance. These data basically correspond to the results of investigations by other authors [1]. We may, therefore, assume that the considerable and fairly protracted changes of the microphone effect which had taken place after a brief local cooling were caused by reflex influences originating upon cold-stimulation of the receptors of the ear drum, tissues of the external auditory canal, or the mucous membrane of the middle ear cavity. As a result, there was a rapid change in the functional condition of the cochlear receptor devices which generate the electric potential, the change lasting a fairly long period of time (3½-4 hours). The non-uniform effect of these influences at various sonar frequencies also corresponds to the "mosaic" character of the reflex regulation of the functional state of the cochlear receptor devices.

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THE MECHANISM OF RESPIRATORY AND CIRCULATORY REACTIONS INDUCED BY THE INTRODUCTION OF CHEMICAL AGENTS INTO THE MESENTERIC VESSELS

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In the modern concepts of the reflex effect of intravascular injections of medicinal preparations and chemical agents on the functions of the organism the role of vascular receptors still remains insufficiently clarified.

V. N. Chernigovskii [4] observed reflex respiratory and vascular changes, upon the action of chemical agents on the receptors of a vascularily isolated loop of small intestines. Brown and Gray [9] recorded the impulse activity from mesenteric chemoreceptors. A. G. Bukhtiyarov [1], S. M. Leites, G. T. Pavlov, and T. S. Yakusheva [2] reported on the changes of respiration, arterial pressure, and blood sugar level, upon injection of pharmacological substances into mesenteric vessels. The results of these experiments were regarded as the effect of reflex influences from the receptors of mesenteric vessels. O. P. Minut-Sorokhtina and B. Z. Sirotin [3] observed the reactions of respiration, blood circulation, and fluctuations of the number of leucocytes in the blood, upon chemical action on the anterior mesenteric vein isolated from general circulation.

Analysis of the participation of the vascular receptors in the mechanism of reflex effects is hampered in a number of experiments by the fact that substances injected into mesenteric vessels may exert an irritating effect on the vascular receptors, as well as on the receptors of tissues or capillaries.

In the present work we investigated the role of the mesenteric vascular receptors in the mechanism of reflex reactions of the respiratory and cardiovascular systems, upon injection of pharmacological substances in these vessels.

METHOD OF EXPERIMENTS

Four series of experiments were carried out on 40 cats under urethan intravenous narcosis. Respiration, arterial pressure, and the rhythm of cardiac activity were recorded.

A loop of the small intestine, isolated from general blood circulation, was perfused with a Ringer-Locke solution. The direction of the flow of the nutrient fluid in the intestinal loop vessels alternated. Under a "direct" perfusion of the organ, the Ringer-Locke solution entered the intestinal loop vessels through the artery and flowed out of the organ through the vein. In "reverse" perfusion, the nutrient fluid entered through the vein and flowed out via artery. Chemical agents were introduced into the stream of the Ringer-Locke solution in "direct" perfusion through a cannula inserted into the artery; in "reverse" perfusion – through the cannula inserted into the vein of the intestinal loop.

In the next series of experiments we studied the latent period of reflex reactions of respiration and circulation upon feeding chemical agents to the receptors of the intestinal loop via "direct" or "reverse" perfusion. In both types of perfusion, an equal volume of Ringer-Locke solution passed through the vascular system in a given unit of time. The rate of flow of the nutrient fluid in the loop vessels was regulated according to the rate of drops of the solution flowing out of the organ.

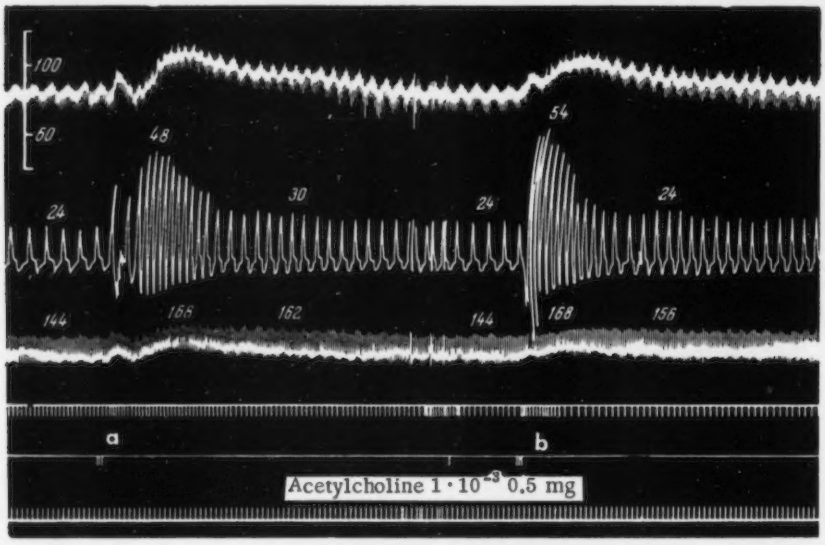

Fig. 1. Character of reflex respiratory and circulatory reactions upon feeding a chemical agent to the receptors of a segment of the small intestine in "direct" and "reverse" perfusion. Designation of curves (top to bottom): arterial pressure; respiration; rate of cardiac contractions; drop-recorder mark; stimulation mark; time mark (one second). Explanation in text.

In our final series of experiments, the chemical agents were introduced into the loop vessels through needles inserted into one of the small branches of the artery and vein. The needle was inserted in the branch of the vessel at the point of the passage of the mesenterium into the intestinal loop. Here, under "direct" perfusion of the organ, the chemical agent was introduced via the needle into a small branch of the vein and, carried by the flow of nutrient fluid, irrigated the venous receptors and moved toward the cannula inserted in the principal vein. In "reverse" perfusion in this case, the chemical agent was introduced via needle into a small arterial branch; it irrigated the arterial receptors and flowed out through the cannula inserted in the principal artery of the loop. Thus, in this series of experiments, the pharmacological substances, injected into the ramifications of the vessels, acted on the vascular walls, but did not enter the capillaries of the loop.

RESULTS OF EXPERIMENTS

Upon feeding 0.5 mg of acetylcholine to the receptors of the intestinal loop in "reverse" perfusion, we observed a pressor reaction, dyspnea, and tachycardia (Fig. 1, a). Feeding of the chemical agent to the loop receptors in "direct" perfusion was accompanied by an analogous reflex effect (Fig. 1, b).

However, feeding of 0.5 mg acetylcholine to the loop receptors in "reverse" perfusion caused dyspnea and hypertension within eight seconds (Fig. 2, a, b), whereas feeding it in "direct" perfusion produced a reflex effect within five seconds (Fig. 2, a', b').

Introduction of 0.5 mg of nicotine through a needle, inserted into a small branch of the vein in the loop, produced no change in respiration and circulation (Fig. 3, a). Introduction of 0.5 mg of nicotine through a

cannula inserted into the loop artery was accompanied by an increase of arterial pressure and dyspnea (Fig. 3, b). Introduction of 0.5 mg of nicotine through a cannula inserted into the vein produced the same effect (Fig. 3, c). Introduction of 0.5 mg of nicotine through a needle inserted into a small branch of the loop artery caused no change in respiration and arterial pressure (Fig. 3, d). Thus, the action of chemical agents on the blood vessel walls does not lead to reflex reactions of the respiratory and cardiovascular systems.

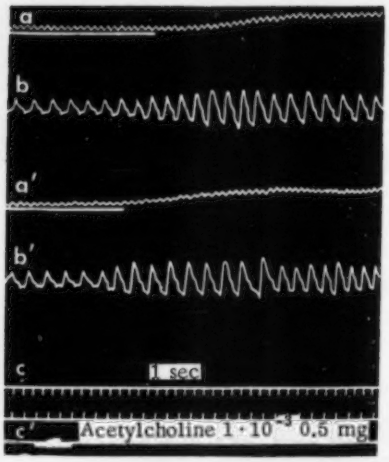

Fig. 2. Latent period of reflex effects upon feeding of chemical agents to the loop receptors in "reverse" and "direct" perfusion. a) Arterial pressure; b) respiration; c) drop-recorder mark; a') arterial pressure; b') respiration; c') drop-recorder mark. Explanation in text.

Thus, the function of chemical irritation shows no reflex reaction on the respiratory and vascular system's centers.

The difference in the duration of the latent period of reflex reactions of respiration and circulation upon chemical action on the receptors of the loop in "direct" and "reverse" perfusion can be explained by the greater rate of the propulsion of the chemical agent to the reflexogenic zone via arterial system, than via venous system.

These experiments demonstrated that the action of chemical agents on the receptors of an organ in "direct" and "reverse" perfusion not infrequently produce entirely analogous reflex effects. The similarity of reflex responses of the receptors of an organ to a chemical stimulation in both types of perfusion has already been pointed out by V. N. Chernigovskii [8].

The identity of reflex reactions, the difference in the length of the latent period when the chemical agents are fed to the receptors of the perfused organ through the arterial and venous bed, as well as the absence of reflex reactions in an isolated action on the mesenteric vessels, permit us to localize the reflexogenic zone of the organ within the limits of capillary and tissue receptors.

There were also no reflex influences observed from the mesenteric vessel receptors in experiments, where chemical agents colored with methylene blue had been injected into the vascular ramifications of an isolated intestinal loop. It was noted in these experiments that the appearance of the colored material on a scarcely noticeable area of the surface of the intestinal loop, from which the blood was washed off with the nutrient solution, was accompanied by fairly well marked reflex effects.

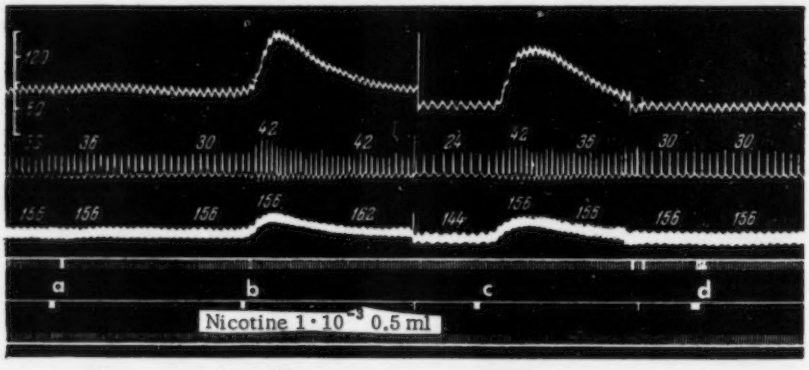

Fig. 3. Absence of reflex effects upon isolated chemical irritation of the artery and vein of the loop of the small intestine. Designations the same as in Fig. 1. Explanation in text.

V. N. Chernigovskii [5, 6] connects the emergence of reflex reactions, upon intravascular injection of chemical agents, with the stimulation of tissue receptors.

It is possible that by prolonged and strong chemical action on the vascular walls one can produce reflex effects similar to those observed by O. P. Minut-Sorokhtina and B. Z. Sirotin [3]. However, the interpretation of these data requires further investigation, since certain substances may stimulate vascular receptors indirectly, as a result of the changed tonus of the vascular wall.

Thus, upon injection of chemical agents into the mesenteric artery, reflex influences on respiration and circulation originate from the receptors of the intestinal tissues. Injection of chemical agents into the mesenteric vein stimulates the hepatic receptors. Chemical irritation of the receptors of these organs is accompanied by stimulation of the respiratory and vasomotor centers.

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OPTIMAL TYPE OF IMPULSES FOR THE ELECTROSTIMULATION OF THE HEART

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Original article submitted March 21, 1960

In recent years electrostimulation of the heart has found employment in clinical practice in cases of its arrest due to a complete block of atrioventricular conductivity, or the absence of automatsim [4, 10, 11, 12]. The effectiveness of electrostimulation in various causes of cardiac arrest has been extensively studied experimentally [6, 7, 10]. However, the problem of optimal form and duration of electric impulses used in electrostimulation has been much less elucidated. As regards the duration of impulses, it should be pointed out that there are considerable variations (from one to 100 milliseconds) in the experiments of various authors [3, 5, 6, 8, 9].

In studying the effectiveness of stimulation of the heart in the arrest of its activity in dogs, poisoned with proserine or cooled to 20°, we investigated the liminal voltage which induces an extrasystole at various lengths and forms of electric impulses. The results of these measurements are described in the present article.

METHODS OF EXPERIMENTS

Experiments were carried out on dogs under usual thermal conditions (18 tests) and under hypothermia (10 tests). The animals of the first group were given a preliminary subcutaneous injection of a 2% pantopone solution, eight mg of the dry substance per kg body weight. Within 40 minutes we separated the femoral vessels under local novocaine anesthesia – the artery for recording arterial pressure, and the vein for the injection of proserine. Proserine was given in the form of a 0.05% solution at 0.5 ml, repeatedly until the onset of considerable retardation of the cardiac rhythm. In four experiments we did not inject proserine, because the cardiac rhythm proved to be sufficiently retarded after pantopone administration.

The animals of the second group received simultaneously with pantopone 0.1 mg/kg of atropine subcutaneously. Prior to, and during the cooling period, a nembutal solution (0.2%) was administered intravenously. The cooling was effected by immersing the dogs into an ice bath. When the body temperature reached 20°, the cooling was terminated and the animals were placed into a water bath of 45° temperature. The warming was stopped when the body temperature reached 33°.

After the cardiac rhythm had been retarded under the effect of proserine or cooling, we carried out electrostimulation of the heart. Needle-electrodes were inserted subcutaneously on the right and left side of the thorax along the location of the heart. We tested isolated monophasic rectangular impulses (from one to 100 msec) and serrated (5 to 10 msec) electric impulses with the aid of a GRAKh-1 apparatus (universal stimulator manufactured by the experimental construction bureau of the AMN SSSR in 1954). We also tested isolated bi-phasic impulses of rectangular and sinusoidal forms. The first were obtained from a source of continuous current (an anode battery with voltage up to 100 V) by means of a mechanical commutator; the other – by means of a discharge of a condenser of 500, 1500, and 3000 miorofarads through a special induction coil without an iron core, calculated for an impulse duration up to 10 msec.

The effectiveness of the electric stimuli was determined by the appearance of an extra pulse wave on the recorded arterial pressure curve.

RESULTS OF EXPERIMENTS

The experiments demonstrated that the magnitude of liminal voltage which produces an extrasystole depends on the form of the electric impulse and on its duration. The lowest threshold was obtained in acting upon the heart with monophasic impulses of rectangular form. At 10 msec duration, the threshold for a monophasic impulse of rectangular form was approximately 1.7-2-fold lower than the threshold for a serrated or sinusoidal form (Tables 1 and 2).

TABLE 1

Liminal Voltage for Rectangular Monophasic and Serrated Electric Impulses

No. of experiment	Liminal voltage (in V)			
	for rectangular impulses lasting		for serrated impulses lasting	
	5 msec	10 msec	5 msec	10 msec
6	31	22	90	60
9	91	70	100	100
12	45	31	70	70
13	–	27	–	45

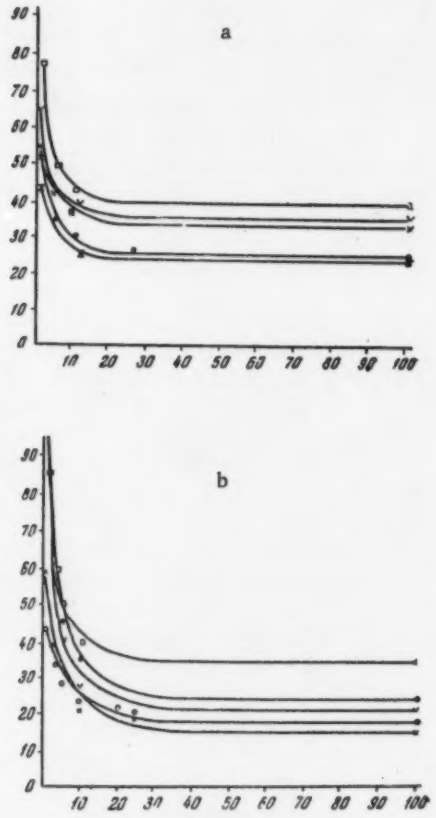
TABLE 2

Liminal Voltage for Rectangular Monophasic and Bi-phasic Electric Impulses of 10 msec Duration

No. of experiment	Liminal voltage (in V)	
	for rectangular monophasic impulses	for rectangular bi-phasic impulses
14	25	24
15	26	30
16	37	48
17	30	33
18	38	53

The liminal voltage for a bi-phasic impulse of rectangular form proved to be equal to a monophasic impulse of the same form.

The liminal voltage for monophasic rectangular impulses was in inverse ratio to their duration. This relationship is graphically presented in the figure.



Dependence of liminal voltage on the impulse duration. a) Curves prepared according to the data of five experiments carried out under usual thermal conditions; b) curves prepared according to the data of five experiments carried out under hypothermia conditions. Along the axis of abscissa – time (in milliseconds), along ordinate axis – voltage (in V).

The figure shows that the point of inflexion of the curves, prepared according to the data of experiments conducted under usual thermal conditions, corresponds to the impulse duration of 7-10 msec (see Fig., a). The inflexion point of curves prepared according to the data of experiments conducted under conditions of hypothermia (see Fig., b) corresponds to impulse duration of 10-13 msec).

The greater effectiveness of monophasic rectangular impulses as compared to sinusoidal and serrated impulses, which we had observed in our experiments, finds its explanation in a certain regular dependence of the electric stimulation effect on the rate (gradient) of increase of the strength of current. As regards the isolated electric impulse effects on the heart, the greater effectiveness of rectangular as compared to sinusoidal impulses was pointed out by Callaghan and Bigelow [3].

In the conducted experiments we were unable to achieve any added stimulating cardiac effect of both phases in the action of isolated bi-phasic impulses of a rectangular current; the liminal voltage for such an impulse proved to be equal to that of a monophasic impulse. In a previously conducted study of the comparative action of an alternating sinusoidal and a rectified (monophasic pulsating) current, under prolonged and continuous cardiac stimulation, we observed the summary effect of both phases—the threshold for the alternating current was twice as low as the one for a rectified current [1]. The stronger stimulating action of an alternating sinusoidal current can be explained apparently by the fact that each of its periods exerts a stimulating effect corresponding to its full amplitude voltage. The absence of a summary effect with an isolated bi-phasic impulse indicates the peculiarities of adaptation changes in the tissues under the effect of stimuli of various duration and quantity.

The dependence of liminal voltage on the duration of the impulse was determined in an indirect stimulation of the heart. Nevertheless, the dependence relationship of this curve is analogous to the one obtained under conditions of direct cardiac stimulation. The need of more prolonged cardiac stimulation in producing an extrasystolic contraction under hypothermia conditions corresponds to the dependence of the duration of the stimulation process on the temperature [2].

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EFFECT OF THE UNCONDITIONED FOOD REFLEX ON THE RHYTHM OF CARDIAC ACTIVITY UNDER PATHOLOGICAL CONDITIONS (AGAINST THE BACKGROUND OF DIPHTHERIA INTOXICATION, ACUTE DISTURBANCES OF CORONARY CIRCULATION, AND PHARMACOLOGICAL EFFECTS)

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In the clinical and experimental investigations by a number of authors [1, 4, 8, 10, 15, 21] the possibility has been established of marked changes of cardiac activity under the effect of reflexes from the organs of the digestive tract.

The effect of food reflexes (conditioned and unconditioned), simulated feeding, swallowing movements, and hunger contractions of the stomach on the rhythm of cardiac activity has been studied by many investigators [2, 9, 11, 12, 13, 22, 23]. It was observed that the food reflex causes an acceleration of the rhythm of cardiac contractions. Direct stimulation of the stomach may exert its effect on cardiac activity, depending on the character of action. Introduction of warm water into the gastric cavity causes a slight deceleration of the cardiac rhythm [6].

Under conditions of cardiac denervation, physiological and emotional reactions, including those induced by food stimuli, are not accompanied by changes in the cardiac rhythm [11, 12].

Of special interest is the problem of the effect of food reactions on the cardiac rhythm under conditions of pathological cardiac activity. There have been cases encountered in the clinic where the vomiting reflex was accompanied by a normalization of the impaired rhythm of cardiac activity [3, 7, 14].

Cases have been described where swallowing movements provoked disturbances of the cardiac activity and, inversely, where they contributed to the normalization of cardiac activity [16]. G. F. Lang [7] pointed out that the nature of these influences is still obscure.

Our work has been carried out with the aim of tracing the effect of unconditioned food and drink reflexes on the rhythm of cardiac activity under various types of its disturbance.

METHODS OF EXPERIMENTS

Experiments were conducted on dogs. Changes of the rhythm of cardiac activity connected with the food reflex were studied on healthy animals, animals with isolated N. vagus, isolated sympathetic cardiac innervation, and in various types of cardiac arrhythmia.

Diphtheria intoxication was produced via single and fractional injections of the toxin in nonlethal and lethal doses. The maximum duration of the disease with a lethal outcome following a single injection of the diphtheria toxin exceeded a month. Strophanthin poisoning was induced via repeated intravenous administrations of the preparation.

Disturbances of coronary circulations were obtained via application of 3 to 5 ligatures to the large branches of the coronary arteries.

The electrocardiograms were recorded by means of "Siemens" and "EKP" electrocardiographs and the "4 PFD" ink-recording device.

In selecting the food stimuli we considered the possibility of a marked reduction of food excitability of the animals in the course of the disease, and employed strong stimuli in our experiments (meat and meat-sugar powder, sausage products, sour cream, sugar, and milk). In cases of marked reduction of food excitability the animals often drank water greedily.

RESULTS OF EXPERIMENTS

Over 1000 observations of dynamic changes of the cardiac activity rhythm in the course of food reactions of healthy animals against the background of normal cardiac activity indicate a monotype character of these changes. During the first few seconds of the unconditioned food reflex the cardiac rhythm becomes accelerated. The extent of acceleration depends on the food excitability of the animal. At a high food excitability, the cardiac rhythm rate at the beginning of the food reflex exceeds by 150-200% the initial rate. The maximum acceleration takes place during the first 6-9 seconds, less frequently, in the first 12 seconds of the food reflex. Starting with the 6th to 12th second, the rhythm of cardiac contractions slows up perceptively, but remains above the initial level up to the end of the food or drink reflex.

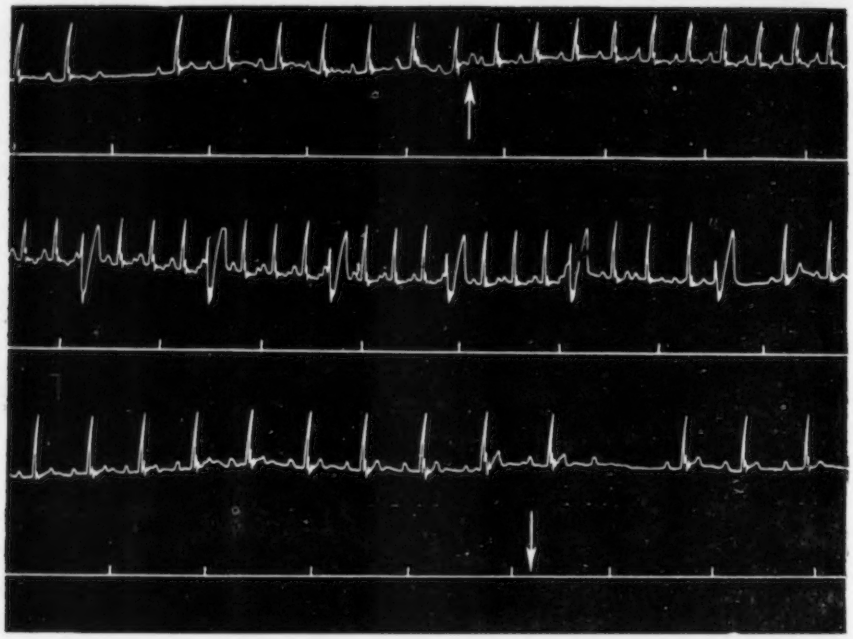


Fig. 1. Changes of the rhythm of cardiac activity in the course of an unconditioned food reflex against a background of prolonged, chronic strophanthin poisoning (the dog drinks milk). The start and termination of the reflex are indicated by arrows. Prior to the start of the unconditioned reflex and following its termination, an incomplete atrioventricular stage II block is noted. The food reflex is accompanied by acceleration of the rhythm of cardiac contractions, improvement of atrioventricular conductivity, and the emergence of ventricular extrasystoles at the height of maximal acceleration of the cardiac rhythm. Designation of curves (from top downward): EKG; annotation of time (one second).

Experiments with food reflexes against a background of partial denervations of the heart were staged on 12 dogs.

The desympathization of the heart was performed by separating as much as 10 pairs of the anterior radicles of the thoracic segments of the spinal cord, starting with the 8th cervical. Following this intervention, the cardiac rhythm acceleration at the start of the food reflex became less pronounced. The cardiac components of the food reflex did not revert to the initial figures until 9 months after the operation.

After a high separation of N. vagus on the neck, the reduction of food excitability and dispeptic phenomena (eructation of food, etc.) hamper the study of the cardiac components of the food reflexes. Against the background of marked reduction of food excitability in the vagotomized animals, perceptible changes of the cardiac rhythm may be absent in the course of the food reflex. When a higher food excitability of vagotomized dogs was present in the course of the food reflex, a distinct, though slight, acceleration of the cardiac rhythm by 10-20 contractions per minute was observed.

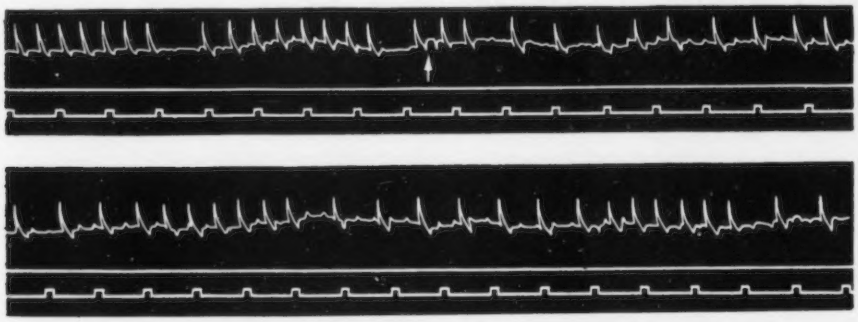


Fig. 2. Acceleration of the atrial rhythm and worsening of atrioventricular conductivity along the path of the unconditioned drink-reflex against the background of severe strophanthin poisoning. The start of the reflex is indicated by an arrow. Designation of curves—as in Fig. 1.

Following exclusion of N. vagus via subcutaneous injection of atropine (from 2 to 4 ml of 0.1% solution), any subsequent acceleration of the cardiac rhythm in the course of a food reflex is possible only at a fairly high food excitability of the animal (we used milk in these experiments). Within 25-30 minutes after atropine injection, with the background of cardiac rhythm at 215 contractions per minute, we observed its acceleration to 250 per minute at the start of the food reflex, and to 270 contractions against a background of 250. (The rate of cardiac rhythm is always indicated in our recalculation per contractions per minute, though in reality we speak of rhythm changes at the start of the food reaction for time segments of 12 seconds' duration).

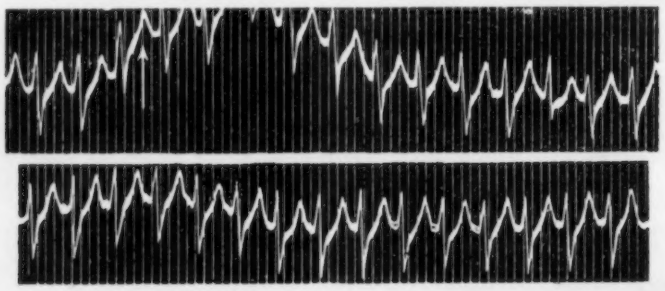
In diphtheria intoxication (experiments on 9 dogs), during the first days of the disease the cardiac components of the unconditioned food reflex are well expressed. Against a background of stage II of an atrioventricular block at the start of the disease and in the course of an unconditioned food reflex we observed a distinct improvement of conductivity.

This fact indicates the important role of the neurogenic component in the disturbances of atrioventricular conductivity in the early stages of diphtheria.

During the subsequent course of the development of the disease, against the background of marked acceleration of cardiac contractions, the food reflexes are accompanied only by slight changes of cardiac activity analogous to its changes in vagotomized animals.

In chronic strophanthine poisoning (observations on 6 dogs), the changes of the cardiac activity rhythm in the course of an unconditioned food reflex depend on the stage of development of the pathological process.

In the most severe forms of intoxication, against a background of a paroxysmal ventricular tachysystolia, the food excitability of the animals was markedly reduced, whereas the drink reflexes induce no distinct changes of the rhythm under these conditions. In less severe forms of intoxication against the background of an incomplete stage II atrioventricular block, the food reflex is accompanied by an acceleration of the rhythm of cardiac contractions most clearly expressed during the first few seconds of the unconditioned reflex. In the majority of instances this is accompanied by a distinct improvement of atrioventricular conductivity and a normalization of the cardiac rhythm. However, diverse variants are possible, depending on the degree of intoxication.



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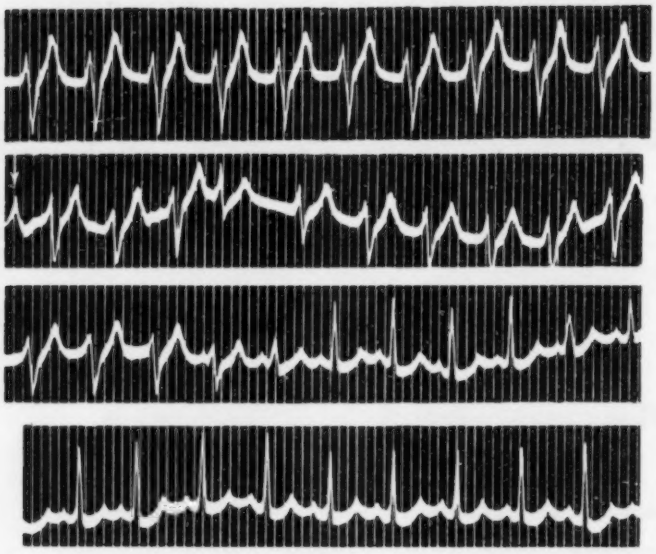

b

Fig. 3. Unconditioned food reflexes against the background of acute disturbances of the coronary circulation. a) Unconditioned food reflex within 24 hours after ligation of the coronary arteries against the background of a severe ventricular tachysystole (240 contractions per minute) is not accompanied by substantial changes of the cardiac activity rhythm; b) unconditioned food reflex in the same dog within 48 hours after ligation of the coronary arteries against the background of ventricular tachysystole (170 contractions per minute) is accompanied by normalization of the cardiac rhythm within a few seconds after the start of the reflex. The start of the unconditioned food reflex is marked with an arrow.

In one of our dogs which has been receiving toxic doses of strophanthin for six years, the unconditioned food reflex (the dog drank milk) was accompanied not only by an acceleration of the sinus rhythm and improvement of conductivity, but also by the emergence of ventricular extrasystoles. They appeared during the first half of the food reaction, at the height of the maximal acceleration of the cardiac rhythm. The extrasystoles in this dog disappeared after the first 12 seconds of the unconditioned food reflex (Fig. 1).

The emergence of ventricular extrasystoles simultaneously with a maximal acceleration of the sinus rhythm and an improvement of atrioventricular conductivity offers a basis for the conclusion that they are connected with an increased tonus of the sympathetic cardiac nerves at the start of the food reflex. The food reflex revealed under these conditions latent foci of intensified activity of heterotopic ventricular automatism.

Acceleration of the sinus rhythm and the improvement in atrioventricular conductivity in the course of the food reflex must be connected principally with the reduction of the tonus of cardiac centers of N. vagus. We observed the improvement of conductivity during the unconditioned food reflexes in five out of six dogs. We encountered an opposite phenomenon in only one of our dogs at the height of severe strophanthin poisoning. A marked acceleration of the atrial rhythm was accompanied in the course of the drink reflex not by an improvement, but by a worsening of atrioventricular conductivity (Fig. 2). In our opinion, this should be regarded as the manifestation of profound parabiotic changes in the conducting system of the heart: the acceleration of impulses which were directed toward the atrioventricular node intensified the parabiotic state of the latter.

In acute disturbances of coronary circulation (experiments on nine dogs), the changes in the rhythm of cardiac activity along the path of food and drink reflexes depended on the time elapsed since the ligation of the coronary arteries and on the initial background of cardiac activity. In paroxysmal ventricular tachysystole of over 200 contractions per minute, the unconditioned food and drink reflexes were not accompanied by substantial changes of the cardiac activity rhythm (Fig. 3, a).

In paroxysmal ventricular tachysystole under 200 contractions per minute, the food and drink reactions were accompanied by distinct changes of the cardiac rhythm. The heterotopic ventricular rhythm changes to a regular sinus rhythm within a few seconds after the start of the unconditioned food reaction (Fig. 3, b).

At a low activity of the foci of heterotopic ventricular automatism against a background of solitary or group ventricular extrasystoles, within 3-8 days, less frequently two days following ligation of the coronary arteries, the unconditioned food reflex produced at once a normalization of the cardiac rhythm in connection with the increase in the activity of the sinus node.

In order to explain the mechanism of the effect of the food reflex on the cardiac activity rhythm in various types of its disturbances, it is expedient to compare the observed changes of cardiac activity with the peculiarities of its regulation at various stages of the pathological process.

The literature [5, 20] and our previous reports [17, 18, 19] point out the effect of extracardial nerves' separation on the initial functional state of the cardiac activity. We observed a stable normalization of the cardiac rhythm resulting from the separation of N. vagus in the disturbances of atrioventricular conductivity and in ventricular rhythms of relatively low frequency. We observed no normalization of the cardiac rhythm, if N. vagus had been separated against the background of ventricular tachysystolia with a rate of over 200 contractions per minute. Analogous results were elicited in the study of the effect of the food reflex on the heterotopic ventricular rhythm.

In evaluating the character of changes of the cardiac components of food reflexes after surgical and pharmacological interventions on cardiac nerves, as well as the peculiarities of the effect of these reflexes on the cardiac rhythm in various types of disturbances, one arrives at the conclusion that the changes of the rhythm of cardiac contractions in the course of a food reflex are connected principally with the reduction of the tonus of the central centers of N. vagus. An increased tonus of the sympathetic cardiac nerves is clearly manifested during the first few seconds of the unconditioned food reflex if the animal possesses sufficiently high food-excitability.

The results of our investigation indicate the possibility of utilizing food and drink reflexes as harmless physiological tests which would permit us to ascertain the peculiarities of the neural regulation of impaired cardiac activity, and to a considerable extent reveal the possibility of its normalization under the effect of surgical neural intervention.

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THE IMPORTANCE OF PROLONGED FUNCTIONAL STRESS OF THE NERVOUS SYSTEM IN THE DEVELOPMENT OF LIPOIDOSIS IN THE AORTA AND MYOCARDIAL LESIONS IN RABBITS

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The successes in the study of atherosclerosis have become possible due to the creation of a model of that illness in rabbits [1]. Studies of this disease in animals and simultaneous morbid anatomical studies on human subjects enabled N. N. Anichkov [2, 3], K. G. Volkova [3, 4], V. D. Tsinzerling [12], and other Russian scientists as well as a number of foreign authors [16 et al.] to study the morphogenesis of atherosclerosis and the importance of exogenous cholesterol in its development. Exogenous cholesterol, however, does not play the same role in human pathology as in the reproduction of the disease in animals. The studies of S. S. Khalatov [8, 9], N. T. Shutova [13, 14, 15], P. D. Gorizontov [5], and other authors who underlined the role of endogenous cholesterol in the development of cholesterolaemia were of great importance for the understanding of the pathogenesis of atherosclerosis. According to these authors, endogenous cholesterolaemia develops in case of disorders of the nervous system. Reports have been published according to which atherosclerosis occurs more frequently and takes a more severe course in persons engaged in mental work [7] or with a history of prolonged psychical traumas [6].

Comparative Data Concerning the Total and Free Blood Cholesterol Level, Aorta Lipoidosis, and Cardiosclerosis During Functional Stress of the Nervous System (Without Administration of Cholesterol)

Animal	No. of rabbit	Average blood cholesterol level				Number of conditioned stimulations	Positive responses (in %)	Lipoidosis of the aorta	Cardiosclerosis	Duration of the observation (in months)
		control estimation		during functional stress of the nervous system						
		total cholesterol (in mg%)	free cholesterol (in %)	total cholesterol (in mg%)	free cholesterol (in %)					
Experimental rabbits	11	32.6	30.3	33.8*	38.0	1800	80.9	–	+	23
	13	36.7	25.1	46.7	33.6	1938	83.6	+	+	23
	15	44.4	25.1	51.1	33.6	1512	90.0	+	+	23
	17	80.0	27.0	72.2	27.1	1578	97.8	–	–	23
	19	22.2	22.7	23.0	43.0	1872	76.4	–		14
	22	68.5	20.6	35.6	42.0	1662	59.3	+		14
Control rabbits	12	31.4	28.0	Not investigated				–	–	23
	14	25.3	29.0					–	–	23
	16	31.4	26.1					–	–	23

* The findings refer to the average cholesterol level before the development of neurosis (see text).

In the present study an attempt was made to establish the influence of prolonged functional stress of the higher parts of the nervous system upon cholesterol and lipoprotein metabolism in the development of atherosclerosis.

METHOD OF EXPERIMENTS

Two series of experiments were carried out on ten experimental and nine control rabbits.

In the first series the total cholesterol was investigated in the serum of the experimental rabbits No. 1, 2, 3, and 4 during functional stress of the nervous system. Later the experimental rabbits (No. 1, 2, 3, and 4) and the control rabbits (No. 5, 6, 7, and 8) were given, against a background of stress of the nervous system, cholesterol in milk for 70 days (0.5 g daily). After an interval of three months the experimental rabbits No. 1 and 4 were given, against a background of stress of the nervous system, and the control rabbits No. 9 and 10 were given 19 egg yolks each for 38 days.

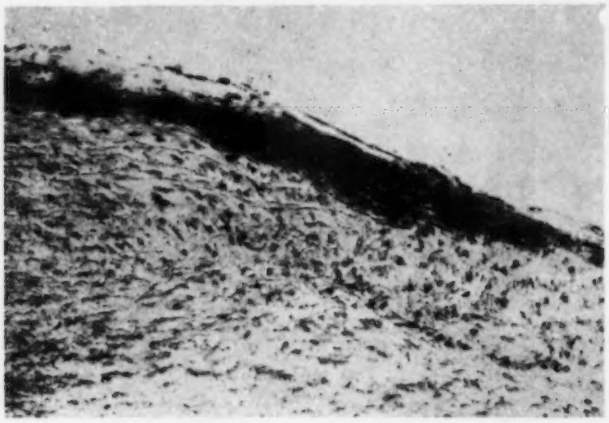

Fig. 1. Aorta wall of a rabbit (No. 13) which had not been given cholesterol with the food. In the abdominal part diffuse deposits and small droplets of lipids can be seen under the endothelium which in some places infiltrate the media. Microphotograph. Staining with Sudan III–haematoxylin. Magnification 180 x.

A period of 30-60 days during which the animals were subjected to experiments, in which a conditioned reflex based on protective inhibition of breathing [10] was evoked, was regarded as a state of stress of the nervous system. Periods of functional stress alternated with periods of rest. 90-100 experiments were carried out with each experimental rabbit. The observation was continued for 12 months. The nervous function was evaluated by the number of positive responses given to the conditioned stimulus in relation to the total number of stimuli.

In the second series of experiments stress of the nervous system was produced by a similar method in six rabbits (No. 11, 13, 15, 17, 19, and 22). The observation was continued for 14-23 months in the course of which 180-250 experiments were carried out with each rabbit. During this period the total cholesterol, cholesterol bound to esters and the free cholesterol, the protein fractions and the lipoproteins in the blood serum were estimated twice monthly. The rabbits were given no cholesterol with the food. Proteins and lipoproteins were separated by paper electrophoresis: the protein was stained with amidoschwarz 10 B, the lipoproteins with Sudan black [11]. Three control rabbits (No. 12, 14, and 16) were used to estimate the indices characterizing the lipoprotein metabolism under normal conditions. Towards the end of the investigation the blood pressure was measured (by the acute method) in three experimental and in two control rabbits. All experimental rabbits of the first and second series and the seven control rabbits were killed by air embolism; the heart and the aorta were fixed in 10% formalin. Pieces of the heart were embedded in paraffin and the sections were stained by the method of van Gieson, Heidenhain, Mallory, Weigert, and were impregnated with silver according to Foot. The other part of the sections of the heart and the aorta were cut on the freezing microtome and stained for fat with Sudan III.

EXPERIMENTAL RESULTS

First Series. During the state of functional stress of the nervous system the total cholesterol of the blood in the rabbits decreased in all experimental rabbits by 24-45%. During the rest period the cholesterol returned to the original level and in some animals (Nos. 2 and 3) even exceeded that level by 60-70%.

Moderate feeding of cholesterol during functional stress of the nervous system did not cause a rise in the cholesterol level in three rabbits (Nos. 1, 2, and 4); whereas in one experimental rabbit (No. 3) and in six control rabbits (Nos. 5, 6, 7, 8, 9, and 10) the cholesterol level in the blood increased 10-15 times, reaching 200-600 mg.

Second Series. During the first three days of functional stress of the nervous system the total blood cholesterol increased by 20-30%; including both the level of cholesterol bound to esters and of free cholesterol; the free cholesterol fraction increased to a greater degree. By the 10th-12th day of functional stress of the nervous system the total cholesterol was lower than the original level; by the 30th-60th day the free cholesterol constituted 40-60% of the total cholesterol, in other words showed a relative increase.

Throughout the whole experiment (14-23 months) the average proportion of free cholesterol in per cent was higher in the rabbits than during the periods of rest and higher than in the control animals (see Table).

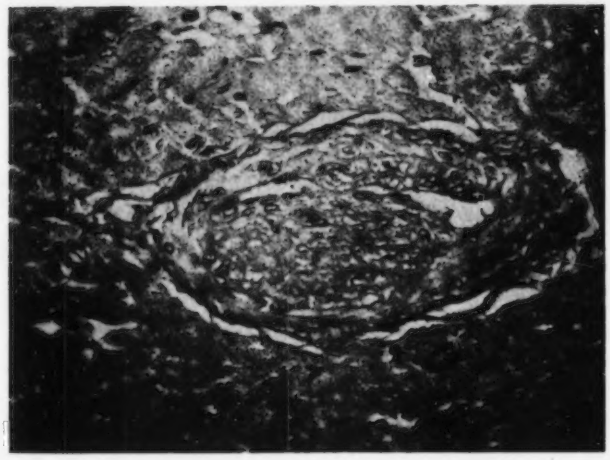

Fig. 2. Section through an artery in the papillary muscle in a rabbit (No. 1). Thickening of the arterial intima on two sides. The lumen of the blood vessel is narrowed. In the thickening course collagenous fibers can be seen among which some histiocytes and fibroblasts occur. At the sites adjacent to the thickening the media is atrophic. Microphotograph staining by van Gieson's method. Magnification 420 ×.

During the stress of the nervous system (30-60 days) the proportion of albumin in the blood serum decreased and the β- and γ-globulin fraction increased. For example, in the control group the average albumin level reached 58.5% ± 0.67, in the experimental group (during the actual experiment) 51.4% ± 1.9 (td = 3.50, in other words the difference between the values was statistically significant)*; in the control group the level of the β-globulins

* $td = (M_1 - M_2)/(\sqrt{m_1^2 + m_2^2})$

reached on the average 12.5% ± 0.33, in the experimental group it increased to 15.7% ± 0.76 (td = 3.89, i.e., the difference was statistically significant); in the control group the γ-globulins reached on the average 16.9% ± 0.67, in the experimental group an increase of the γ-globulins to 20.7% ± 0.67 could be observed (td = 4.0, i.e., the difference was statistically significant).

The level of α-lipoproteins and β-lipoproteins in the experimental rabbits No. 19 and No. 22 decreased during the functional stress of the nervous system, which lasted 30-60 days. During the rest interval the level of α-lipoproteins and β-lipoproteins increased. Towards the end of eight months' work with continued stress of the nervous system the reaction was reversed and an increase in the level of β-lipoproteins and neutral fats could be observed (colored spot in the region of the γ-globulins). In experimental rabbits No. 11, 13, 15, and 17 lipoproteid investigation results came after 17 months' work. In addition, the strain on the nervous system was watched for growth of β-lipoprotein and neutral fats.

Towards the end of the investigation a neurosis developed in experimental rabbit No. 11: during that period the blood cholesterol level increased in this rabbit within seven days from 25 to 420 mg% out of which 300 mg% consisted of free cholesterol. The level of β-lipoproteid and neutral fats showed a similar marked increase. The blood pressure in the experimental rabbits and the control rabbits varied between 98 and 112 mm Hg, i.e., the blood pressure was normal.

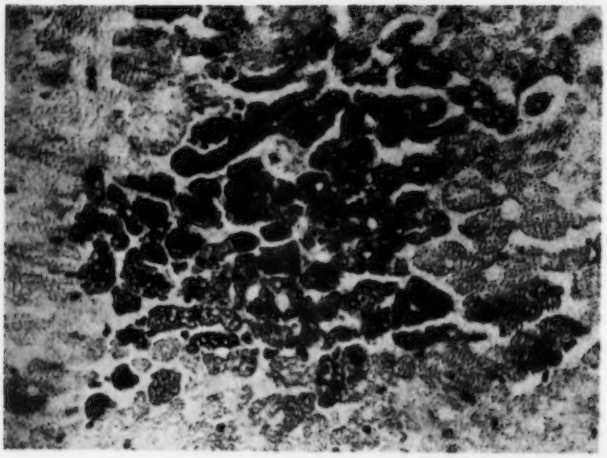

Fig. 3. Section through the papillary muscle of a rabbit (No. 11). Part of the muscle in a state of necrosis. Vacuoles of different size can be seen in the muscle fibers. Microphotograph staining by Heidenhain's method. Magnification 380 ×.

Histological investigation revealed in two experimental rabbits (Nos. 1 and 4) which had been given cholesterol with the food and had a normal blood cholesterol level the presence of lipoidosis of the aorta. In experimental rabbit No. 3 and in two control rabbits in which the blood cholesterol level had varied between 300 and 600 mg% lipoidosis of the aorta could also be observed. In the aorta of the experimental rabbits the lipids were situated along the internal elastic membrane under the endothelium in the shape of diffuse deposits and small droplets.

In the group of experimental rabbits which had never been given cholesterol but the nervous system of which had been exposed to prolonged functional stress lipoidosis of the aorta also developed in three out of six animals (see Table).

In the rabbits No. 13 and No. 15 the deposits of lipids were situated in the abdominal part of the aorta, under the endothelium along the internal elastic membrane (Fig. 1); in rabbit No. 22 in the beginning part of the aorta also under the endothelium along the internal elastic membrane. The lipids appeared in the shape of diffuse deposits and small droplets. In rabbits No. 13 and No. 22 they infiltrated the media of the aorta adjacent to the internal elastic membrane (see Fig. 1).

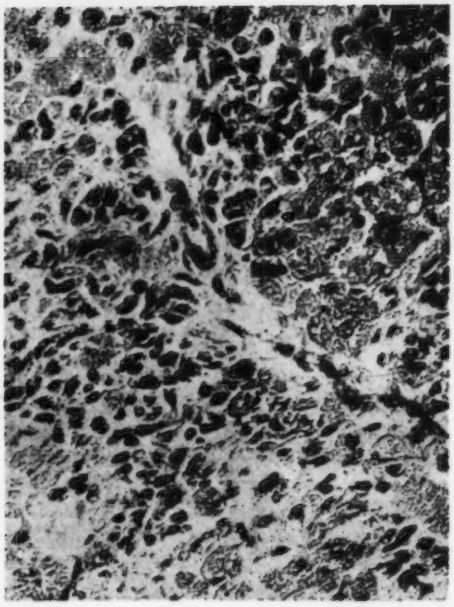

Fig. 4. Section through the left ventricle of a rabbit (No. 11). Accumulation of polyblast elements and fibrocytes which include necrotic muscle fibers. Microphotograph. Staining by van Gieson's method. Magnification 380 ×.

In the rabbits No. 13, 15, and 22 the blood cholesterol level never exceeded 80 mg%; a relative increase in the level of free cholesterol and an absolute increase in the level of β-lipoproteins, and neutral fats could be observed. In three other experimental rabbits (Nos. 11, 17, and 19) and control rabbits (Nos. 12, 14, and 16) no deposits of lipids could be observed in the aorta.

In the coronary arteries of the experimental rabbits (Nos. 1, 2, and 4) which had been given cholesterol and of the experimental rabbits (Nos. 11, 13, and 15) which had not been given cholesterol with the food, thickening of the arterial intima could be observed. In the coronary arteries of the control animals no thickening of the arterial intima could be observed.

Thickenings of the arterial intima were mainly situated in the arterioles and occurred more frequently in rabbits which had been given cholesterol. In sections stained with Sudan III no lipids deposits could be found in the thickenings. Only in one rabbit (No. 15) a yellow stain along the course of the elastic fibers could be seen in a changed area of the arterial intima in a large branch of the left coronary artery. The thickenings were produced by fairly coarse collagenous fibers arranged without any definite order which included some histiocytes and fibroblasts (Fig. 2). In places adjacent to the thickening the media of the blood vessels was atrophic (see Fig. 2), the internal elastic membrane was split up and interrupted. Besides a great number of argyrophile fibers could be seen in the thickenings.

In sections through the myocardium the capillaries were found to be moderately dilated and congested with blood in all experimental rabbits. In the myocardium of the experimental rabbits Nos. 1, 2, 4, 11, 13, and 15* small areas were found in which the muscle fibers were of homogenous appearance or were in a state of necrotic fragmentation.

In the papillary muscles of rabbit No. 11 necrotic areas could be seen (Fig. 3) in other regions of the myocardium accumulations of polyblast elements and fibrocytes, including necrotic fibers, could be seen (Fig. 4).

Analysis revealed that the severity of the myocardial lesions was related to the duration of stress to which the nervous system was exposed and to the character of the conditioned reflex activity. In rabbit No. 11 which was suffering from neurosis the myocardial lesions were most severe of all.

SUMMARY

Lipoidosis of aorta with a thickening of the tunica intima of the coronary vessels and dystrophy of the cardiac muscle was seen to develop in prolonged functional stress of the nervous system (12-23 months) in rabbits which received food with or without cholesterol.

Lipoid deposition into the wall of aorta occurred with the cholesterol blood level equal to 35-70 mg%; however, the content of free cholesterol, β-lipoproteids, and neutral fats was found to be increased in the blood of experimental rabbits. The mentioned lesions in the aorta, the coronary vessels of the heart, and the cardiac muscle are the sequence of prolonged functional stress of the nervous system.

* The state of the myocardium in rabbits Nos. 19 and 22 was not investigated.

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SOME PROBLEMS CONCERNING THE REFLEX REGULATION OF THE VASCULAR TONUS IN ANAPHYLACTIC SHOCK

COMMUNICATION II. CHANGES OF THE DEPRESSOR REFLEXES DURING THE LOWERING OF THE ARTERIAL BLOOD PRESSURE CAUSED BY ANAPHYLACTIC SHOCK

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(Presented by Active Member, Academy of Medical Sciences, USSR, N. N. Gorev)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 5, pp. 44-48, May, 1961
Original article submitted June 25, 1960

In a previous communication [9] we had shown that vascular pressor reflexes are not only restored during the lowering of the arterial blood pressure during anaphylactic shock but become even stronger compared to the normal level and we also showed that these changes in the pressor reflexes cannot be explained with the development of a state of parabiosis in the vasomotor center.

These findings suggest that in anaphylactic shock of moderate severity the capacity of the vasomotor center to respond to pressor stimuli was restored or even increases.

It was the aim of the present paper, to study the changes of vascular depressor reflexes under similar conditions: the reflexes were investigated in the beginning period of the lowering of the arterial blood pressure caused by injection of the shock dose of the serum as well as during the restoration of the arterial blood pressure.

METHOD OF EXPERIMENTS

The experiments were carried out on 28 rabbits under nembutal narcosis. During the sensitization of the animals the injection of the shock dose of serum as well as the recording of the arterial blood pressure and the respiration were carried out by the method described in the previous communication.

The vascular depressor reflexes were produced by electrical stimulation of rectangular impulses in the aortic nerve or by stimulation of the pulmonary mechanoreceptors produced by increasing the pressure of air within the lungs.

The afferent impulses from the aortic nerve were taken off by means of a loop oscillograph (MPO-2) with an alternating current amplifier and recorded on a cinematographic film. Simultaneously, the arterial blood pressure and the respiration were recorded on the same film.

EXPERIMENTAL RESULTS

Under normal conditions the strength of the depressor response after stimulation of the aortic nerve reached on the average 30-35 mm Hg. Intravenous injection of the shock dose of serum led to a marked fall in the blood pressure, the level of which reached by the 5th-10th minute 40-50 mm Hg. By that time in all experiments without exception a marked suppression of the depressor response could be observed, the strength of which reached on the average 5-11 mm Hg (Fig. 1). Later parallel to the restoration of the general blood pressure the depressor response also began gradually to gain in strength. But even by the 20th minute after the injection of the shock dose of serum when the level of the general blood pressure had reached 60-70 mm Hg, the strength of the depressor response was much lower than under normal conditions. And what is more, in the majority of experiments these reflexes did not regain their normal strength even by the 30th-40th minute. Similar changes in the depressor reflexes could be observed also in the experiments in which the pulmonary mechanoreceptors were stimulated. It must be emphasized that by that time the pressor reflexes had not only been restored but had even become stronger than the reflexes observed under normal conditions.

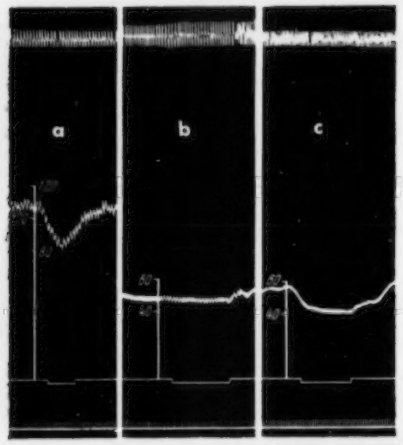

Fig. 1. Vascular depressor reflex in response to stimulation of the aortic nerve in a rabbit. a) Under normal conditions; b, c) 10 and 20 minutes after administration of the shock dose of serum respectively. Significance of the curves: (from top to bottom): respiration; arterial blood pressure; mark denoting the moment of stimulation; time mark (1 sec).

The data quoted above suggest that the capacity of the vasomotor center to respond to the depressor stimuli is much lower under conditions of anaphylactic shock than under normal conditions.

We were unable to find publications concerning the state of the depressor reflexes in anaphylactic shock. Investigations concerning other types of shock suggest that the majority of authors is inclined to believe that changes of the depressor reflexes under these conditions are caused by development of a state of parabiosis in the vasomotor center. For example, V. K. Sel'tser [7] observed, that simultaneously with the development of the state of shock the so-called "rule of strength" is disturbed and in many cases a narcotic levelling, and ultraparadoxical phase can be observed in the changes of the depressor reflexes.

To obtain a paradoxical response from the arterial blood pressure and above all the use of an intensive stimulus is required. For example, it is known that in this manner V. E. Delov and V. I. Filistovich [3], and V. I. Filistovich [8] caused a pessimal state in the depressor mechanisms.

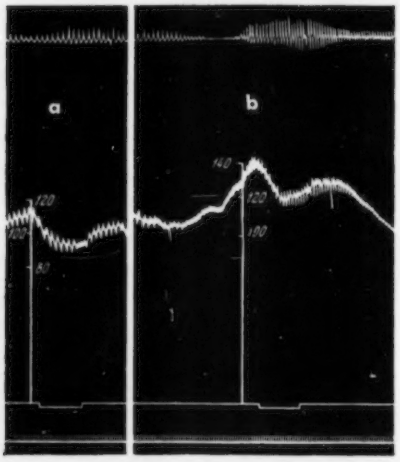

Fig. 2. Vascular depressor reflex in response to the stimulation of the aortic nerve under normal conditions (a) and during the erectile phase of shock development (b). Key as in Fig. 1.

Can it be assumed, however, that the changes in the depressor reflexes, described above, are connected with extreme stimulation of the depressor mechanisms strong enough to produce a state of parabiosis in the central part of the reflex arch of the depressor reflexes? A stimulation of the depressor mechanisms may well take place during the intravenous injection of the shock dose of serum as well as during the erectile phase of the shock when a temporary increase in the arterial blood pressure can be observed, a fact which will increase the stimulation of the baroreceptors in the region of the carotid sinus and the aortic region. Some experiments, however, suggest that even if such stimulation actually takes place its intensity would be so insignificant that it could never produce a pessimal state in the central part of the reflex arch of the depressor reflexes. The kymogram (Fig. 2) shows that additional stimulation of the intact aortic nerve during the erectile phase of the shock at the time when the baroreceptors undergo temporary stimulation by the increase in the arterial blood pressure, even without additional stimulation, causes a depressor response of similar strength as those observed under

normal conditions. To this purpose we investigated with the aid of a loop oscillograph the afferent impulses passing through the aortic nerve under normal conditions at the time of intravenous injection of the shock dose of serum and during the development of the shock. It appeared that the injection of the shock dose of serum has almost no influence upon the intensity of the afferent impulses passing through the depressor nerve (Fig. 3). A slight increase in these impulses could be observed immediately after the injection of the serum, but this increase was to a large extent caused not so much by the action of the shock dose of serum as rather by the increase in the arterial blood pressure caused by the development of the erectile phase of the shock.

No appreciable intensification of the afferent impulses could be observed in the aortic nerve during the state of shock. On the contrary, the electrogram (Fig. 3, c) shows that the intensity of the afferent impulses decreases correspondingly to the decrease in the general arterial blood pressure.

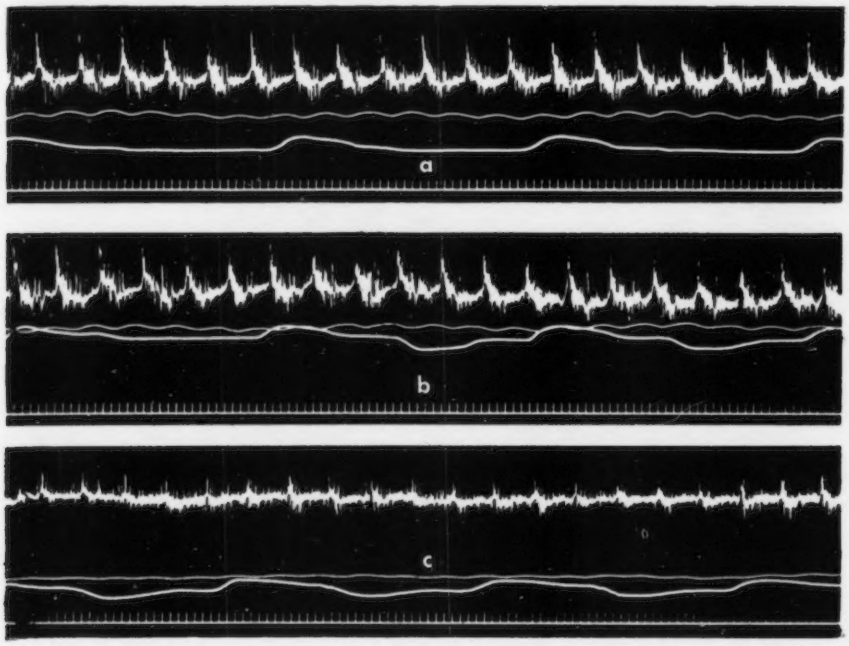

Fig. 3. Rhythmically recurring afferent impulses in the depressor nerve of a rabbit. a) Under normal conditions; b) towards the end of the intravenous injection of the shock dose of serum; c) correspondingly in the 15th minute of hypotension. Significance of the curves (from top to bottom): afferent impulses in the depressor nerve; arterial blood pressure; breathing; time mark (1/20 secs), zero level of the arterial blood pressure.

The appearance of oscillations of small amplitude in the interval between the individual discharges is probably due to the stimulation of the chemoreceptors in the aortic arch consequent to the hypoxia produced by the fall in the arterial blood pressure.

It thus appears that although the intensity of the afferent depressor impulses increases temporarily during the development of the shock state, this increase is so insignificant that it could under no circumstances produce a pessimal state in the central part of the reflex arch of the depressor reflexes.

It can be assumed that similar changes in the strength of the afferent impulses take place under the conditions described above in the nerves of the carotid sinus. In this context some authors [1, 2]were unable to obtain under normal conditions a pessimal reaction in the arterial blood pressure even after super-strong stimulation of the depressor nerves. On the other hand, reports appeared in the literature suggesting that a distortion of the depressor reflexes can be obtained even by relatively weak stimulation of the depressor mechanisms [10, 11, 12].

The distortion of the depressor reflexes after weak threshold stimulation of the aortic nerve was observed in the experiments of A. G. Sverdlov [6] under conditions of lower blood pressure produced by traumatic shock.

Finally, we wish to emphasize that both the decrease in the capacity of the vasomotor center to respond to depressor stimuli observed under conditions of shock on the one hand and the fall in the strength of the afferent impulses in the depressor nerve on the other hand have a favorable influence upon the rapid restoration of the arterial blood pressure. And, indeed, as I. R. Petrov [5] remarked: "In what state would be the animal body if any stimulation of the depressor mechanisms would lead to a fall in the arterial blood pressure which is already at a critical level." For this reason, the suppression of the depressor mechanisms under these conditions appears to be to a certain degree of adaptive character.

SUMMARY

Experiments were performed on rabbits and dogs. In conditions of anaphylactic shock of moderate intensity depressor reflexes are markedly inhibited at the beginning of shock-induced arterial hypotension. Later, with the restoration of arterial pressure these reflexes become intensified, but there is no complete normalization. Investigation of the afferent depressor impulsation during shock development, along with that of depressor reflexes against the background of shock (its erectile phase) shows that the mentioned inhibition of depressor reflexes is evidently not caused by the development of the pessimal state to depressor actions in the vasomotor center.

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THE INFLUENCE OF RESECTION OF TWO-THIRDS OF THE STOMACH UPON THE RESORPTION OF SODIUM PHOSPHATE (P^{32}) FROM THE DIGESTIVE TRACT AND ITS UTILIZATION IN THE ANIMAL BODY

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In an earlier publication [2] we were able to show that disorders of the resorption of breakdown products of casein labelled with I^{131} and methionine labelled with S^{35} in the digestive tract can be observed in dogs after resection of the stomach according to Billroth II in the modification of Finsterer. These changes were due to disorders of the evacuatory function of the stomach stump.

After the resection of the stomach the resorption shows changes immediately after as well as at later periods after the operation. The observation was continued for a period between one and 16 months after the operation.

With the aid of radioactive sodium phosphate (P^{32}) used as indicator we collected an extensive experimental material which characterized the resorption capacity of the digestive tract in various functional states: during the stimulation of the n. vagus and the receptor zone of the stomach; during blockage of the vegetative nervous system with atropine, after transsection of the n. vagus, in anemia, caused by denervation of the fundus and corpus and under other conditions [2, 3, 4]. A fact established by us in an earlier investigation: changes in the resorption of phosphate from the intestine and in its utilization in the body in pathological conditions of the liver ought to be particularly emphasized.

There are reports in the literature according to which stomach resection creates conditions for the development of a pathological process in the liver. In view of this fact, we found it necessary to study on one and the same animal at various periods after the stomach resection the resorption of phosphate from the intestine and to compare the changes found with those changes which had been found by us in cases of hepatitis.

The present investigation was carried out simultaneously with the investigations concerning the resorption of breakdown products of casein labelled with I^{131} and methionine labelled with S^{35} on the same dogs [5].

METHODS OF EXPERIMENTS

Seven dogs were used for the experiments. In five of them stomach resection by the method of Billroth II in the modification of Finsterer was carried out. The rate of resorption of sodium phosphate ($Na_2 HP^{32}O_4$) and its utilization in the animal body were initially studied in control experiment before the resection of two-thirds of the stomach and then at various periods after the operation. The longest period of observation lasted 16 months.

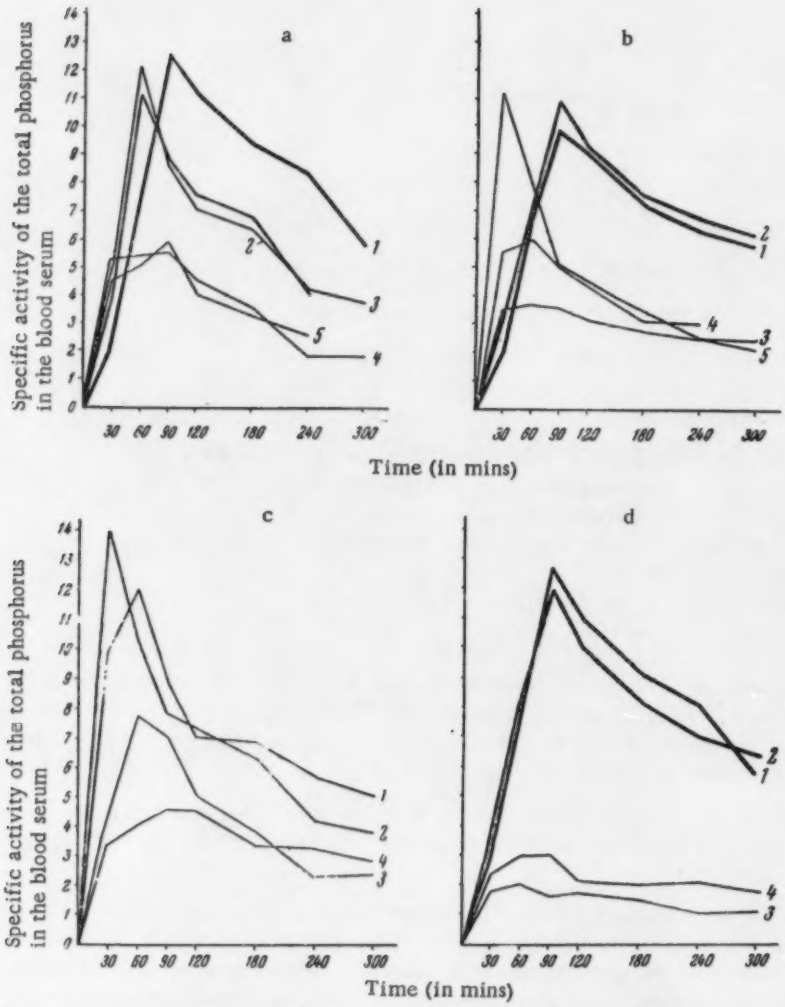

Fig. 1. Rate of resorption of P^{32} from the digestive tract and its utilization under normal conditions and at various times after stomach resection. a) The dog Rus: curve 1) Normal conditions; after resection of the stomach: curve 2) after one month; curve 3) after three months; curve 4) after seven months; curve 5) after eleven months; b) the dog Veta: curves 1 and 2) Normal conditions; after resection of the stomach: curve 3) after three months; curve 4) after ten months; c) the dog Silacs after resection of the stomach: curve 1) after one month ten days; curve 2) after three months ten days; curve 3) after six months; curve 4) after 12 months; d) the dog Naf: the curves 1 and 2) normal conditions; after resection of the stomach: curve 3) after four months; curve 4) after 14 months.

Radioactive sodium phosphate ($Na_2 HP^{32}O_4$) in a dose of 100-200 counts per 1 g weight was given to the dogs on an empty stomach in a mixture of milk and water (total volume 150-200 ml). The blood was taken at strictly observed intervals after the administration of P^{32} (after 30, 60, 90, 120, 180, 240, 300, and 360 mins). The total phosphorus content and the radioactivity of the blood serum were estimated. The specific activity of the total phosphorus of the blood serum was estimated by the method described in earlier publications [4].

EXPERIMENTAL RESULTS

Altogether 24 experiments were carried out.

In the first period after the resection of the stomach (after two-four months) a more rapid resorption of P^{32} from the digestive tract could be observed – a fact indicated by the shift of the peak of radioactivity in the blood serum to the left, i.e., nearer to the moment of injection of P^{32}. Here the radioactivity of the blood decreased more rapidly than under normal conditions (Fig. 1, a, b, c).

The more rapid resorption of P^{32} from the intestine into the blood observed in the first period after the resection of the stomach is connected with the accelerated evacuation from the stomach taking place in these dogs after the operation (see previous paper) [5]. In a subsequent series of experiments carried out to confirm this conclusion P^{32} was ingested into the digestive tract of the dog Tsyganka, (the stomach of which animal was intact,) by two methods: perorally and through a duodenal fistule. Fig. 2 shows a shift to the left in the peak of the specific activity of the total phosphorus and inorganic phosphorus in the blood serum after the second method of ingestion of P^{32} (directly into the gut).

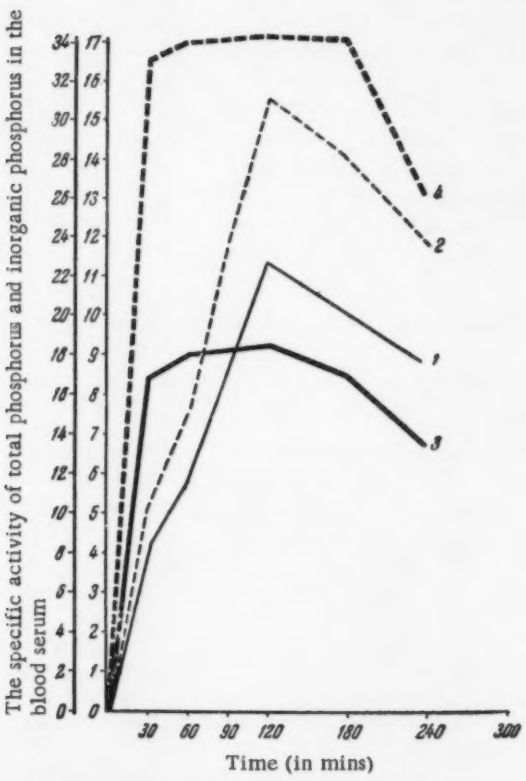

Fig. 2. The dog Tsyganka. The specific activity of total phosphorus (curves 1 and 3) and of inorganic phosphorus (curves 2 and 4) in the blood serum after injection of P^{32} by different methods: curves 1 and 2) per os; curves 3 and 4) through a duodenal fistule. The scale of inorganic phosphorus has been reduced to one half of the scale used for the total phosphorus in the blood serum.

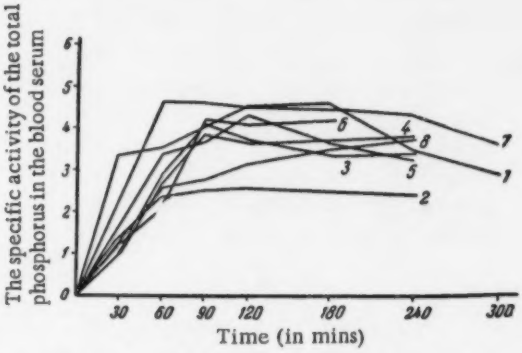

Fig. 3. The rate of resorption of P^{32} from the digestive tract and its utilization in dogs suffering from hepatitis. Curve 1) Igra; curve 2) Atos; curve 3) Belka; curve 4) Kroshka; curve 5) Kerzon; curve 6) Malyshka; curve 7) Mops; curve 8) Klyaksa.

At later periods marked changes take place in the character of resorption and utilization of sodium phosphate. Two-four months after the stomach resection the curves characterizing the specific activity of the total phosphorus in the blood serum become, with regard to their character, more similar to those obtained in dogs suffering from pathological conditions of the liver (Fig. 1, a, b, c, d).

By comparison, we present in Fig. 3 earlier findings concerning the resorption and utilization of P^{32} in dogs known to be suffering from liver lesions.

The findings obtained by us warrant the conclusion that stomach resection leads to disorders in the liver function. This conclusion was confirmed by histological investigation.

N. A. Nilova carried out histological investigation of the liver tissues in three dogs within 6-12 months after stomach resection. In the dog Silacs atrophic liver cirrhosis was found. In the dogs Jack and Rus vacuolization of the liver cells of varying degree could be found. In some cases the nuclei of the liver cells were pycnotic, the liver parenchyma was infiltrated with leucocytes and lymphocytes, the intralobular venous capillaries between the strands of liver cells were narrowed, and other changes could be seen.

In the papers of I. M. Lipets [8], I. T. Kurtsin [6], and L. I. Belorybkina [1], the authors emphasize the close functional interrelation between the stomach and the liver. There are also reports in the literature concerning pathological changes in the liver and bile ducts after stomach resection [9, 10, and others].

In our experiments it could be established that notwithstanding the more rapid evacuation of food from the stomach stump in the dogs at various periods after the operation the resorption of P^{32} from the intestine decreased in later periods. How can we explain the fact that the character of resorption of protein breakdown products and phosphate changes at various periods after the operation?

The pathological process in the liver which gradually develops after stomach resection apparently has a different influence upon the amino-acid metabolism and inorganic phosphorus metabolism in the liver. It may be that the processes of protein synthesis in the liver* are affected to a lesser degree and the rate of phosphorus metabolism in the liver undergoes more marked changes. For this reason the changes which take place after stomach resection in the rate of resorption and utilization of breakdown products of casein labelled with I^{131} and methionine labelled with S^{35} remain in the period following the operation on one and the same level: the level and the character of the radioactivity in the blood remain unchanged.

SUMMARY

The rate of P^{32} absorption from the digestive tract and of its utilization in the organism was studied at various intervals after subtotal resection of the stomach in 7 dogs. Radiophosphorus was given to dogs per os in water-milk mixture. The content of total phosphorus and its radioactivity were examined in the blood serum at various periods; its specific activity was estimated as well.

The rate of P^{32} absorption from the digestive tract was increased and a maximal rise of blood radioactivity was shifted to the left in dogs with subtotally resected stomach (as compared to the control animals). With the passage of time the character of P^{32} absorption from the digestive tract was changed in the animals with subtotally resected stomach; the rate of P^{32} utilization from the blood was altered as well. Specific activity of the blood became similar to that in dogs with parenchymatous hepatitis. P^{32} absorption remained altered at remote time periods after the resection of the stomach (the longest period of observation—16 months), being evidently connected with the structural changes in the liver occurring in these conditions.

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* S. M. Leites and G. T. Pavlov [7] observed, in rats afflicted by severe liver lesions, marked changes in the rate of protein synthesis in the liver.

ENZYME-SECRETING FUNCTION OF THE INTESTINE IN DOGS, AS INFLUENCED BY ADRENALECTOMY AND INJECTION OF SOME STEROID HORMONES

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For a long time there has been evidence to support a close connection between the condition of the adrenal cortex and the activity of the digestive tract. For instance, the well-known impairment of digestive activity during Addison's disease provides such evidence.

It is widely held that hormones of the adrenal cortex play an important role in the development of general adaptive reactions of the organism to strong nonspecific stimulants. In particular, these hormones can protect the digestive system from the damaging effects of such stimulants. It is also known that hormones of the adrenal cortex can influence a number of important metabolic processes in tissues. In this connection, their influence on the enzyme-secreting function of the intestinal mucosa is of special interest. The mucosa rapidly synthesizes various substances, including highly specific enzymes, and is distinguished under normal conditions by a high rate of regeneration of the epithelium, which is sloughed off into the intestinal lumen during the process of secretion [2, 5, 6].

We attempted to study the enzyme-secreting function of the intestine in dogs, as influenced by adrenalectomy and by making up hormonal deficiencies with parenteral injection of cortisone and deoxycorticosterone (i.e., representatives of the glucocorticoids and mineralocorticoids); and to clarify whether there are any differences in the effects of these two hormones on the intestine under set conditions.

EXPERIMENTAL METHODS

The experiments were carried out on three dogs, in which two isolated loops were formed, one from the duodenum and the other from the lower part of the large intestine (by Thiry's method). After the original level of secretion had been re-established, first one adrenal was removed, and secretion was observed; then, approximately thirty days after the operation, the second adrenal was removed. Afterwards the dogs were given daily intramuscular injections of steroid hormones to maintain vitally important functions: first cortisone and deoxycorticosterone, then cortisone alone, both hormones again, and finally deoxycorticosterone alone. Each period consisted of 15-20 days.

We selected the doses of these hormones in preliminary experiments. By trying out increasing quantities of hormones, we settled on those quantities which produced a good general state of the animals, good utilization of food, normal motility, and absence of visible impairment of digestive activity; in other words, we settled on those quantities of hormones which prevented appearance of clinically expressed symptoms of adrenal insufficiency.

In a period when only one hormone was being injected, the dose of cortisone per kg body weight was 0.41 mg, and the dose of deoxycorticosterone acetate was 0.068 mg. In a period when both hormones were being injected, the dose of cortisone was 0.26 mg, and the dose of deoxycorticosterone was 0.03 mg, per kg.

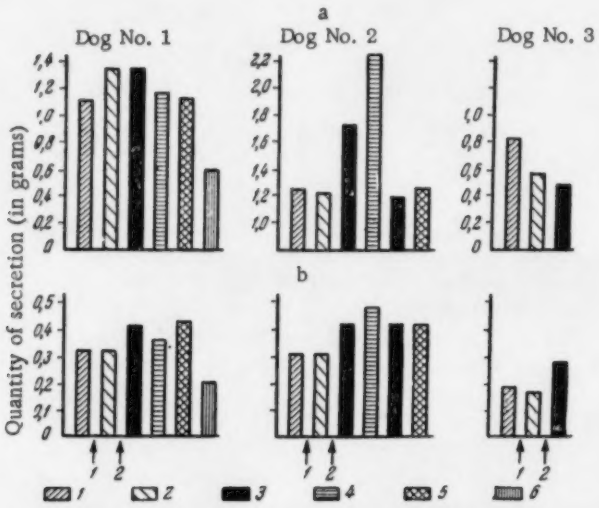

Fig. 1. Quantity of secretion obtained from isolated intestinal loops in one hour. a) Total quantity; b) dense fraction. Arrow 1) Removal of one adrenal, arrow 2) removal of both adrenals. 1) Before adrenalectomy; 2) after removal of one adrenal; 3) after removal of both adrenals and with injection of cortisone and deoxycorticosterone; 4) injection of cortisone; 5) injection of deoxycorticosterone; 6) without hormones.

In external appearance and behavior these dogs did not differ from dogs on which no operations had been performed. Throughout the whole experiment the animals received the usual full amount of food ration. When cortisone alone was being injected, they received in addition 5 g of sodium chloride.

Intestinal secretion in the dogs was studied regularly three times a week. Intestinal juice was collected over the course of five hours during intermittent secretion. The amounts of the fluid and dense fractions were determined quantitatively; then the secretion was homogenized, and the homogenate was analyzed for amounts of the enzymes, enterokinase by the method of G. K. Shlygin [4], alkaline phosphatase by splitting of sodium phenolphthalein phosphate [3], saccharase by splitting of cane-sugar (using a polarimetric method), and peptidase by splitting of peptone, followed by titration of carboxyl groups with 0.2 N potassium hydroxide in a 90% alcohol solution (for further details see [1]).

EXPERIMENTAL RESULTS

After removal of one adrenal (without supplementary injection of hormones) the quantity of secretion (in particular, its dense fraction) obtained from isolated loops of the intestine in dogs underwent no significant change.

However, the quantity of some enzymes (computed per unit weight of the secretion) fell noticeably. This was particularly true of enterokinase and saccharase, whose concentrations decreased in some dogs by 40-50%. On the other hand, the content of peptidase (total determination) showed a tendency to increase in some dogs (Figs. 1 and 2).

After removal of the second adrenal, followed by regular injection of cortisone and deoxycorticosterone, changes were observed both in enzyme secretion and in the quantity of intestinal juice (see Fig. 1). The total

quantity of juice generally increased. Thus, for instance, the quantity of juice from dog 1 rose on the average from 1.12 to 1.37 g per hour. One's attention is particularly caught by the increased secretion of the "dense" fraction of intestinal juice, consisting primarily of epithelial cells sloughed off by the mucosa. The secretion of this fraction of intestinal juice rose significantly (for instance, in dog 1, from 0.32 to 0.42 and sometimes to 0.62 g per hour). In Fig. 1 these changes are illustrated by average values, computed from 5-10 determinations in each experimental period.

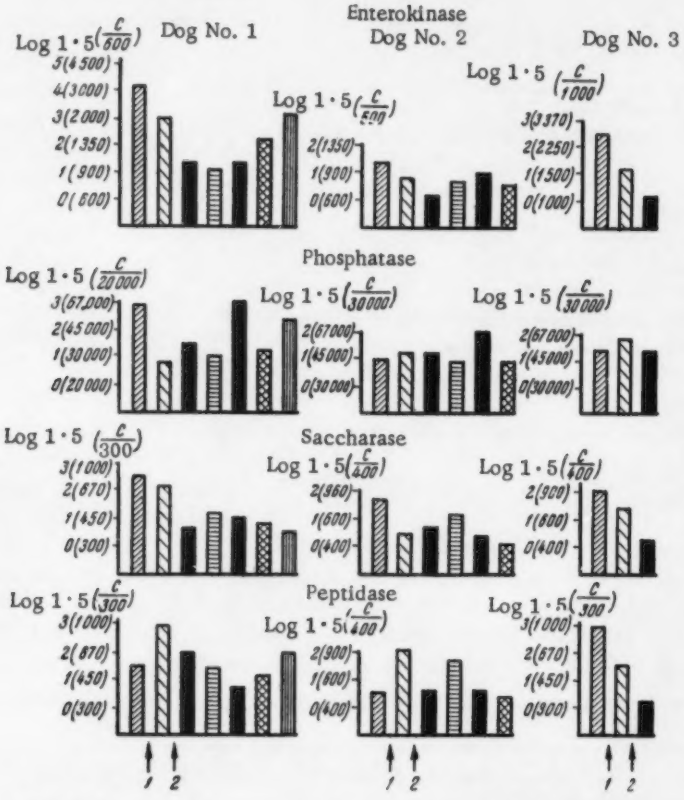

Fig. 2. Content of enzymes (in relative units) in 1 g of the "dense" fraction of intestinal secretion. Symbols same as in Fig. 1. c) content of enzyme.

We also determined the amount of nucleic acid in the collected secretion (by the method of Schmidt and Tannhauser). Nucleic acid content per unit weight of the "dense" fraction of juice did not change significantly, and this indicates that the relative content of cellular elements in this fraction did not change. Hence, it is probably that the increased amount of the "dense" fraction is connected with more rapid sloughing off of the intestinal epithelium.

Changes in enzyme content of the secretion after removal of the second adrenal followed the same pattern as after removal of one adrenal, but changes were more sharply expressed. The content of enterokinase (per g of secretion) declined by a factor of more than 2, in comparison with the original level (Figs. 2 and 3). The saccharase content also decreased sharply, while the concentration of alkaline phosphatase frequently remained unchanged and declined in only one dog. The content of peptidases was close to the level in the original period. Despite the increased secretion of the "dense" fraction of juice, the quantity of enterokinase and saccharase secreted per hour underwent a significant decrease (Fig. 3).

These changes in secretion of juice and enzymes were basically confirmed and extended when cortisone alone or deoxycorticosterone alone was injected, instead of both hormones. Some slight differences were that saccharase content in the secretion gradually increased during injection of cortisone alone, and decreased again during injection of both hormones (see Fig. 4). The content of alkaline phosphatase was somewhat higher in the period of application of both hormones than in periods of injection of either hormone alone (see Fig. 2).

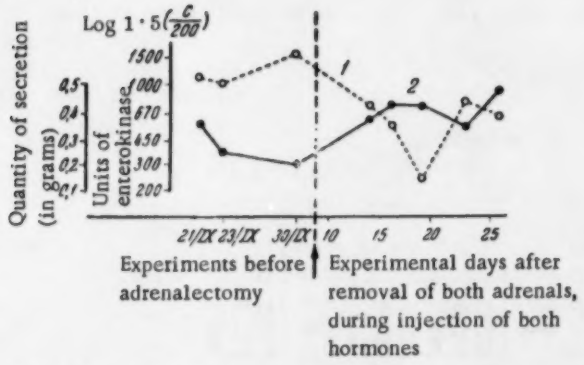

Fig. 3. Changes in the quantity of the "dense" fraction of the secretion and the content of enterokinase in this fraction (in units per g) after adrenalectomy, during injection of cortisone and deoxycorticosterone. Dog No. 2. 1) Enterokinase content in the "dense" fraction of the secretion; 2) quantity of the "dense" fraction of the secretion; both adrenals were removed at the arrow. c) content of enterokinase.

At the end of experiments on one dog (No. 1), injection of hormones was stopped. At this point the total quantity of juice and, in particular, the quantity of the "dense" fraction increased, especially at the end of the five-day period of observation (see the table). At the same time, the concentration of enterokinase in the secretion declined significantly.

In all dogs blood also appeared in the "dense" fraction of the secretion after removal of both adrenals. In one of the dogs this was distinctly observable, even after removal of one adrenal.

At the end of this series of experiments, when dog No. 2 had been sacrificed and was being dissected, a certain degree of hemorrhage into the mucosa of the small intestine was noticed. This same finding was more apparent in dog No. 1, which was killed five days after injection of hormones had ceased. Dog No. 3 died as a result of sudden appearance of symptoms of cardiac motor failure, a month after removal of both adrenals, during which period she received both hormones. At post-mortem it was noticed that the mucosa of the stomach, small intestine, and large intestine of this dog was saturated with blood. There were no visible changes in other organs. In all dogs a lowered level of enzymes in scrapings from the mucosa of various parts of the intestine was noticed.

Quantity of Juice (in g per hr) and of Enzymes (in Relative Units) After Injection of Hormones had Ceased (Dog No. 1)

Day after injection of hormones had ceased	Juice secreted per hour		Enzymes secreted per hour			
	total quantity	"dense" fraction	enterokinase	phosphatase	saccharase	peptidase
3rd	0.82	0.29	820	12456	113	186
4th	0.74	0.23	614	16906	102	156
5th	0.25	0.10	105	3797	31	–

Thus, removal of one or both adrenals leads to similar changes in the enzyme-secreting activity of the intestine, the chief differences being in the extent of their appearance. In the first case, of course, reduced adrenal function sets in; however, when both adrenals are removed and cortisone plus deoxycorticosterone are injected (together or separately), a certain degree of adrenal insufficiency evidently still arises.

First of all, injection of these two hormones might not be equivalent to their normal secretion, but more important, while these hormones can maintain the general clinical condition of the animals, they cannot replace the whole complex of hormones produced by the adrenal cortex and, therefore, they cannot maintain all functions which are in one way or another influenced by this hormonal complex.

As we have seen, this level of adrenal insufficiency chiefly decreases the output of the most specific extracellular enzymes of the intestine, enterokinase, and saccharase. The least effect is upon secretion of intracellular enzymes, such as peptidase, whose content in the secretion even shows a tendency to increase.

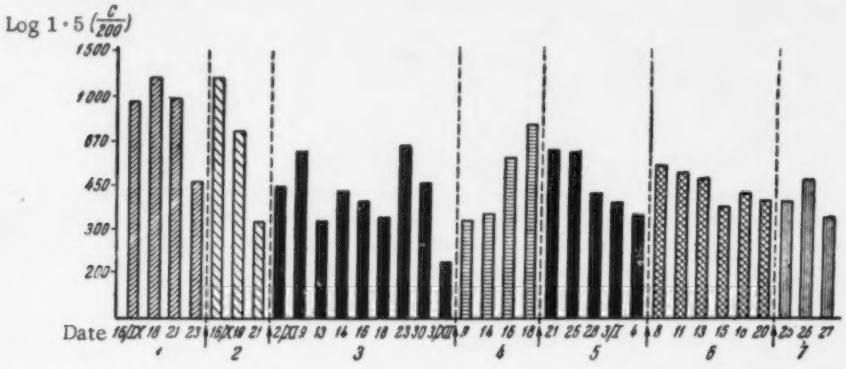

Fig. 4. Saccharase content in the dense fraction of the secretion. Dog No. 1. 1) Before adrenalectomy; 2) after removal of one adrenal; 3) after removal of both adrenals, during injection of the two hormones; 4) injection of cortisone; 5) injection of the two hormones; 6) injection of deoxycorticosterone; 7) without hormone. c) content of saccharase.

We also observed changes in other aspects of secretory activity of the intestinal mucosa, expressed as increased secretion of the "dense" fraction of intestinal juice (basically, epithelial cells sloughed off in the process of secretion) and regular appearance of blood in the "dense" fraction of juice.

These results permit one to conclude that the adrenal cortex plays an important role in regulating a number of aspects of the enzyme-secreting function of the intestine, by its output of hormones; the adrenal cortex stimulates formation and secretion of the most specific intestinal enzymes at a high level, checks secretion of the "dense" (cellular) fraction of juice, and also maintains the integrity of the blood vessels in the intestinal mucosa.

Further experiments are necessary to clarify the mechanism of this effect of the adrenal cortex.

The author is sincerely grateful to Prof. N. A. Yudaev for valuable advice while this study was being carried out.

SUMMARY

As established in experiments on dogs with isolated intestinal sections, the amount of the "solid" portion and the total secretion per time unit exhibited no significant changes after unilateral adrenalectomy; however, the enterokinase and saccharase content in the secretion showed a marked drop. After bilateral adrenalectomy and administration of cortisone and desoxicorticosterone the amount of secreted "solid" portion of the intestinal juice becomes greater, whereas the enterokinase and saccharase content is considerably diminished. There is a tendency towards the peptidase content increase. The main features of these changes are retained if cortisone or desoxicorticosterone alone is administered for a period of 10 days, although in such conditions there occur some variations in the secretion of individual hormones. Without hormone administration the total amount of intestinal secretion and of its "solid" portion, as well as the quantity of enzymes secreted per time unit gradually decreases. After bilateral adrenalectomy blood is always present in the "solid" portion of the secretion.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**

MUSCLE PROTEINS OF BLOOD VESSEL WALLS, AND CHANGES IN THEIR COMPOSITION DURING HYPERTENSIVE DISEASE

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When one analyzes the factors producing a rise in arterial pressure during hypertensive disease, most clinicians and pathologists start from the assumption that changes in the basic level of tension and in the strength of contraction of vessel musculature are chiefly or even exclusively determined by causes external to the vessel wall [10, 11].

In its most general and categorical form, this point of view has been expressed by A. L. Myasnikov [12]: "Rise in blood pressure during hypertensive disease depends upon various factors lying outside the sphere of the vessels."

However, a number of clinical investigators adhere to other views concerning the origin of vessel tension. Thus, V. A. Val'dman [1, 2], while accepting the important role of neuropsychic, hormonal, and other influences on vessel tonus, nevertheless views arterial hypertensive disease as a disease of the vascular system. In this formulation of the nature of the disease, great significance is attached to changes in the vessels themselves during the development of arterial hypertension.

One might suppose that since contraction of vessels is directly connected with the musculature of vessel walls, a comparative study of the fractional composition of vascular muscle proteins under normal conditions and during hypertensive disease could shed some light on this question.

As is well known, the more pronounced is a muscle's ability to contract tetanically and tonically, the higher is its percentage content of proteins of the actomyosin complex.

Unlike the contractile function, the holding function of smooth musculature (unfatiguing resistance to stretch), as shown by numerous investigations of I. I. Ivanov and co-workers [3-7], depends upon proteins of a type distinct from actomyosin. The view formulated by I. I. Ivanov in 1948 concerning the existence of two different substrates of muscular activity – contraction and tonus, and the distinctness of the substrate of unfatiguing tonus from actomyosin – has received further experimental support in recent years from the work of other authors [13-16].

Therefore, the aim of the present study was to investigate the fractional composition of vascular muscle proteins under normal conditions and during hypertensive disease.

EXPERIMENTAL METHODS

For this study we used the renal arteries and their branches, and also muscular arteries of other human viscera.

The vessels were removed from a corpse at the time of post-mortem on the day of death or on the following day, but usually no later than twenty-four hours after death.

From our results and also the results of B. S. Kasavina and Yu. M. Torchinskii [9], the fractional composition of muscle proteins undergoes no change of any significance for twenty-four hours and even for longer periods of time under conditions when the corpse is kept at low temperatures (0-4°). This assumption also receives indirect support from the evidence of V. A. Val'dman and A. A. Nechaev [1], obtained in the laboratory of N. P. Kravkov; these authors found no change in the vascular reactions of isolated organs for many hours after death.

Normal human vessels were obtained from the bodies of people who were not afflicted with diseases of the circulatory system and had died as the results of accidental causes or other reasons.

Corresponding vessels were taken from bodies of people who were afflicted with a severe chronic form of arterial hypertension.

Pieces of vessels were freed from external and internal membranes. Thoroughness of the dissection, as a result of which the middle, muscular strip was isolated, was checked by microscopic observation of the resulting material in a number of cases, especially in the first period of the work.

Tissue of the vascular muscle layer was cut up with sharp scissors and then ground with finely-divided quartz. The resulting homogenate was fractionated by the method described in the work of I. I. Ivanov and coworkers [4]. Total tissue nitrogen, nonprotein nitrogen, and protein nitrogen in all fractions were determined by a micro-Kjeldahl method. The results given below were typical of each experimental series.

EXPERIMENTAL RESULTS

In the first experimental series, we studied the fractional composition of the muscular layer from normal vessel walls.

Vessel musculature differs somewhat in its protein composition from other types of smooth muscle (for instance, stomach or uterus). However, it should be noted that the object of our study was not vascular muscle tissue but the muscular layer as a definite anatomical structure. As is well known, these concepts are not identical, since the middle layer of vessels, apart from the muscular elements, contains a certain and sometimes a very considerable quantity of elastic fibers.

The high content of stromal proteins which we found in vessel wall musculature is closely connected with these details of its histological structure. Nevertheless, this fact could not have significant influence on the quantitative ratios between different fractions of muscle proteins, and in particular, between fractions of myofibrillar proteins.

Results concerning the protein composition of vessel wall muscle are set out in the table.

Besides the high percentage content of stromal proteins, one should note details of the composition of myofibrillar proteins.

Myofibrillar proteins were extracted by Weber's solution from a homogenate of muscle tissue after preliminary exhaustive extraction of sarcoplasmic proteins, and after dialysis against distilled water or very dilute solutions, the myofibrillar proteins could be separated into two fractions: 1) proteins soluble in salt solutions with high ionic strength; under these conditions this fraction precipitated out (the AM fraction); 2) proteins soluble in salt solutions of low ionic strength and found in the supernatant fluid (fraction T, according to the nomenclature of I. I. Ivanov and co-workers [4]).

In detecting connections between protein composition of muscle and details of its function, the greatest interest attaches to results concerning the fractional composition of myofibrillar proteins.

The AM/T ratio in muscle of different types varies noticeably. Skeletal muscle, which is suited to rapid phasic contraction, has a relatively high content of proteins of the AM fraction. On the other hand, tonic smooth muscle has a relatively higher content of myofibrillar proteins soluble at low ionic strength [4].

However, it should be observed that the composition of myofibrillar proteins (fractions AM and T) in different types of muscle is not the same. Thus, if fraction AM in skeletal muscles is almost exclusively actomyosin, fraction AM extracted under analogous conditions from smooth muscle contains, in addition to actomyosin, significant quantities of nucleoproteins and possibly other proteins. The composition of fraction T is also not the same in muscles of different types.

Fractional Composition of Proteins of Vessel Wall Muscle (in percentages of total nitrogen due to nitrogen in the fraction)

Residual nitrogen	Sarcoplasmic proteins soluble in salt solutions with low ionic strength	Myofibrillar proteins				Stromal proteins
		total	soluble in salt solutions with high ionic strength (AM)	soluble in salt solutions with low ionic strength (T)	AM/T	
Vessels of a healthy man						
1,95	10,81	9,94	1,9	7,87	1:4	75,7
Vessels of cattle						
1,49	18,45	9,17	1,35	7,41	1:5	71,42
Vessels of a man suffering from hypertension						
2,79	13,05	9,13	5,18	3,52	1,5:1	73,88

Vessel muscle tissue has a very high relative content of proteins of fraction T. The AM/T ratio in such muscle is approximately 1 : 4, with small variations in individual cases.

We may recall that this quantity is 1 : 1.5 for stomach muscle of the rabbit and 1 : 3 for myometrium [4].

This property of the composition of myofibrillar proteins is evidently closely connected with the ability of vascular muscle to maintain prolonged, unfatigued tonic tension.

It is interesting to observe that the protein composition and, in particular, composition of myofibrillar proteins of the muscular wall of homologous vessels in man and animals (cattle) are rather similar (see the table).

Vessels of animals have a significantly higher relative content of sarcoplasmic proteins.

The following series of experiments was devoted to studying the fractional composition of proteins of vessel wall muscle during hypertensive disease (see the table).

When one compares the results in the table, it is evident that the percentage content of myofibrillar proteins in vessels of patients with hypertension is approximately the same as in normal vessels, but the AM/T ratio in these vessels is completely different. Whereas in normal vessels the predominant part of the myofibrillar proteins consists of proteins of fraction T, while fraction AM, containing the contractile proteins of muscle, represents about 25%, in patients with hypertension the vessel proteins soluble in salt solutions with high ionic strength (fraction AM) represent the greater part of the total myofibrillar proteins.

Patients with hypertension have somewhat higher relative content of residual nitrogen and sarcoplasmic proteins in their vessels. The quantity of stromal proteins under normal and pathological conditions is approximately the same. The absolute content of total nitrogen and the nitrogen of the fractions, calculated per unit weight of dry tissue, shows some scatter in different experiments, since tissue dried out during the dissection to separate the muscular layer of vessels.

From the point of view of greatest interest to us, the results concerning differences in fractional composition of myofibrillar proteins are of the highest significance.

In a study of changes in the composition of proteins of skeletal muscle during paralysis arising from poliomyelitis, I. I. Ivanov, V. A. Yur'ev, and others [8] showed that biochemical changes in contractile proteins provide an objective and highly sensitive index, which permits one to estimate the functional state of the muscles. The observed changes in fractional composition of vessel myofibrillar proteins during hypertensive disease are exactly the reverse of those observed during impairment of motor activity of skeletal muscles due to the paralytic form of poliomyelitis, denervation of muscles, cutting of the tendons, etc.

In evaluating these results, one should, of course, bear in mind that the nature of the contractile reaction in muscles may be influenced not only by the composition of myofibrillar proteins but also by various other factors; but one, nevertheless, gains the impression that hypertensive disease involves not only hypertrophy of cardiac muscle but also an increased contractile ability of vessel wall musculature, and conditions are created for independent hyperfunction of contractile elements.

This impression is confirmed by the results of other authors.

In particular, the ATP-ase activity of myofibrillar proteins extracted from diseased vessels is much higher than in normal vessels, and this also provides evidence for a higher content of the primary proteins of the actomyosin complex.

At present we are carrying out a more detailed study of the fractional composition and enzymatic properties of myofibrillar proteins from normal and pathological vessels.

These changes in the protein composition of vessel musculature can doubtless play an important role in the origin of peculiar vessel reactions, not only to pathological but also to the usual physiological stimulants.

It is quite probable that changes in fractional composition of vessel muscle proteins can take place in other forms of persistent vessel dysfunction as well.

SUMMARY

Fractional composition of protein in the muscular layer of arterial wall was studied in persons without any cardiovascular affection, and in the patients suffering from a severe form of arterial hypertension. There was a relative increase in the content of myofibrillar proteins soluble in salt media of high ionic concentration in the patients with prolonged and severe arterial hypertension. The relationship existing between the shifts in the protein composition of the vascular musculature and the changes in the contractile function of the vessels is discussed.

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SPECIES DIFFERENCES IN THE DISTURBANCES OF CARBOHYDRATE AND ASCORBIC ACID METABOLISM OF RODENTS FOLLOWING ADMINISTRATION OF PLAGUE TOXIN

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We have shown [1, 2] that administration of toxins extracted from strain EV plague microbes leads to a fall in the ascorbic acid content of various organs of rodents. For these experiments we used white mice and rats, which are very sensitive to the plague toxin, and the less sensitive gerbils; we found that certain species differences could be discerned in the effect of the toxin on the vitamin C content of the organs examined. We found that 7-18 times as great a dose of strain EV Pasteurella pestis autolyzate was required to bring about the same degree of depletion of ascorbic acid in the resistant rodents as in rats or mice.

The connection between species differences in resistance of rodents to plague toxin and the intensity of the metabolic disturbances caused by the toxin was even more clearly shown by the results of our investigations of carbohydrate metabolism in plague intoxication. The fall in liver glycogen content of white mice and rats following administration of P. pestis EV autolyzate or fraction II of virulent plague microbes was greater than in guinea pigs, which are highly resistant to plague toxin.

These findings may be of interest not only for the better understanding of the pathogenesis of plague intoxication, but also for the elucidation of the nature of the species differences in sensitivity of animals to plague toxin. For this reason we repeated our earlier experiments on white mice, guinea pigs, and gerbils, including also two more species of rodent, viz., emuranchiks and dwarf gophers, which are more resistant to the action of plague toxin than are white mice.

In view of published reports [7] that ascorbic acid metabolism is closely bound up with that of carbohydrates, our analysis of the effect of plague toxin on the experimental animals included determination of the ascorbic acid contents of the suprarenal glands, spleen, liver, and lungs, as well as of liver glycogen and blood sugar.

EXPERIMENTAL METHODS

The wild rodents were trapped in the steppes of the Stavropol and Astrakhan Regions, as follows: gerbils (Meriones meridianus nogaiorum Heptn.) and dwarf gophers (Citellus pygmaeus Pall.) in the Levokum Province, and emuranchiks (Scirtopoda telum Licht.) in the Volga Province.

The animal material used in the experiments was as follows: 30 white mice (20 males and 10 females, weighing from 19 to 30 g), 20 gerbils (10 males and 10 females, weighing from 38 to 63 g), 20 emuranchiks (10 males and 10 females, weighing from 52 to 72 g), 29 dwarf gophers (14 males and 15 females, weighing from 140 to 300 g), and 21 guinea pigs (17 males and 4 females, weighing from 370 to 800 g).

TABLE 1

Changes in the Contents of Liver Glycogen, Blood Sugar, and Ascorbic Acid in Organs of Rodents 30 Minutes After Intraperitoneal Injection of Fraction II of Pasteurella pestis

Species of rodent	Dose of fraction II (mg/100 g)	Number of animals	Liver glycogen content (%)	Blood sugar content (mg, %)	Ascorbic acid content (mg, %)			
					of suprarenal glands	of spleen	of liver	of lungs
White mice	Control	15	3,51±0,9	127,2±6,8	209,4±56,4	35,8±7,6	32,0±5,3	17,6±7,5
	0.036	15	1,83±0,16	158,8±2,4	124,0±15,8	27,3±3,6	29,3±1,8	11,3±3,6
Gerbils	Control	10	3,71±0,6	119,4±3,9	119,0±28,3	46,3±11,1	27,0±4,4	9,6±1,4
	0.05	10	2,86±0,5	141,5±8,4	71,9±17,1	33,9±7,5	28,1±7,2	8,9±0,6
Emuranchiks	Control	10	5,32±0,97	147,9±12,4	169,0±50,0	99,0±21,5	18,1±4,2	20,1±5,1
	0.05	10	3,75±1,74	167,5±8,7	102,5±13,0	42,5±8,5	29,5±11,0	9,3±2,1
Guinea pigs	Control	12	3,62±0,42	104,7±0,9	74,8±27,6	25,5±5,9	12,5±2,1	9,3±1,0
	0.04-0.06	9	3,8±0,78	118,2±7,8	55,0±15,3	16,7±4,4	7,1±1,5	4,9±2,8
Dwarf gophers (experiment No. 1, October)	Control	10	5,48±1,1	150,3±24,5	114,8±2,5	17,3±4,8	18,5±3,1	9,1±3,8
	0.05-0.06	10	7,83±1,45	154,4±21,8	114,8±24,4	17,6±4,4	21,3±4,9	7,5±3,0
Dwarf gophers (experiment No. 2, April)	Control	4	2,44 (0,95—3,15)	103 (92—118)	105,2 (70,7—144,4)	16,6 (10,3—25,0)	20,5 (13,5—25,6)	10,0 (5,1—14,3)
	0.05	5	1,66 (0,56—3,0)	125,4 (65—145)	44,6 (28,0—77,0)	12,9 (5,1—22,2)	11,1 (6,1—14,9)	5,4 (3,6—6,6)

* The figures in parentheses indicate the range of the values found.

TABLE 2

Species Differences in the Liver Glycogen Content, the Blood Sugar Content, and the Ascorbic Acid Content of Different Organs of Rodents Injected with Plague Toxin

Species of rodent	Degree of change (as % of the value found for the controls)					
	content		ascorbic acid content			
	of glycogen in the liver	of blood sugar	of the adrenals	of the spleen	of the liver	of the lungs
White mice	−47.9	+24.8	−40.8	−23.8	− 8.5	−35.8
Gerbils	−22.9	+18.5	−39.6	−26.8	+ 4.1	− 7.3
Emuranchiks	−29.5	+13.2	−39.4	−57.1	+16.3	−53.8
Guinea pigs	+ 5.0	+12.8	−26.5	−34.5	−43.2	−47.4
Dwarf gophers (experiment No. 1, October)	+42.9	+ 2.7	0	0	+15.1	−17.6
Dwarf gophers (experiment No. 2, April)	−32.0	+22.0	−58.0	−22.0	−46.0	−46.0

Note: − fall, + rise

The experiments on white mice, gerbils, and emuranchiks were performed in the second half of July, on guinea pigs towards the end of September, and on dwarf gophers during the hibernation period (the first half of October), and after hibernation (early April).

The animals of each species were divided into two groups, one of which served as the controls, while the other was given plague toxin. The same numbers of males and females were included in each group.

The toxic material used was fraction II of virulent plague microbes (strain No. 137), prepared in our Institute by M. I. Levi by the method of Baker et al. [5]. The LD_{50} of this fraction, when given by intraperitoneal injection to male white mice, amounted to 0.009 mg, or 0.032 mg/100 g. Freshly prepared solutions of fraction II in 0.85% NaCl were given by intraperitoneal injection into white mice, at a dosage rate of 0.036 mg/100 g, and into the other four species of rodents at the rate of 0.04, 0.05, or 0.06 mg/100 g, according to their weight.

Our earlier experiments showed that plague toxin [1, 3], similarly to other stress-provoking factors [8, 9], caused more or less pronounced response reactions during the first few minutes of their action on the organism. Bearing this in mind, in the present research we killed the animals by decapitation or by a heavy blow on the head 30 minutes after injection of toxin.

Liver glycogen was determined by Pflüger's method, as modified by Osterberg, blood sugar by the Hagedorn-Jensen method, and ascorbic acid by indophenol titration according to Harris and Ray.

EXPERIMENTAL RESULTS

As is shown by the mean values given in Tables 1 and 2, the effect of 30 minutes of action of the plague toxin was to cause a more or less considerable fall in liver glycogen content, a rise in blood sugar, and depletion of the ascorbic acid content of the organs examined.

Does there exist any connection between the intensity and nature of these changes and the species sensitivity of rodents to plague toxin?

The results obtained in our laboratory for the sensitivity of five species of rodents to plague toxin are shown in Table 3. These comparative data for species sensitivity to plague toxin are incomplete, and require further work for the determination of LD_{50} of fraction II for emuranchiks, guinea pigs, and dwarf gophers. Preliminary experiments showed that, as for autolyzates of plague microbes, guinea pigs and dwarf gophers were the most resistant to fraction II: guinea pigs survived doses of 0.5 mg/100 g, and dwarf gophers of 2.5 mg/100 g.

TABLE 3

Sensitivity of Different Species of Rodents to Plague Toxin Given by Intraperitoneal Injection

Species of rodent	Magnitude of lethal dose		
	MLD of Pasteurella pestis EV autolyzate (mg/100 g body wt.)	LD_{50} for fraction II of Pasteurella pestis No. 137	
		(mg per animal)	(mg/100 g body wt.)
White mice	0.2	0.009	0.032
Gerbils	0.3-2.2	0.078	0.18
Emuranchiks	0.4-0.5	–	–
Guinea pigs	1.8-2.4	–	–
Dwarf gophers.	6.0	–	–

A consideration of the data of Tables 1, 2, and 3 reveals the existence of a certain degree of correlation between the sensitivity of a given species of rodent to plague toxin and the extent of glycogenolysis in the liver of the given animals. The most pronounced fall in liver glycogen, with the correspondingly greatest rise in blood sugar content, was found in white mice, which are the most susceptible to the toxin. Much smaller changes were found in gerbils and emuranchiks, even when somewhat higher doses of toxin were injected. Liver glycogen did not fall in guinea pigs, although there was a slight rise in the blood glucose content. Injection of toxin into hibernating dwarf gerbils (experiment No. 1) caused a fairly considerable rise in liver glycogen content, without appreciable change in the blood sugar level. The same doses of toxin administered to the animals after their spring awakening (experiment No. 2) caused pronounced glycogenolysis and hyperglycemia. This seasonal difference in the response of gophers is evidently related to seasonal fluctuations in the state of their carbohydrate metabolism and reactivity [4, 6]. This conclusion is not contradicted by the results obtained with emuranchiks, which also belong to the class of hibernating rodents. They differ from dwarf gophers in entering into hibernation in the late fall, with the onset of the first frosts. The experiments on emuranchiks were conducted in July, when the animals were active.

Our second group of results concerned the effect of toxin on the ascorbic acid contents of different organs. The extent of lowering of ascorbic acid content after 30 minutes of toxemia showed no regular correlation with species sensitivity to toxin. However, the more susceptible species of rodents showed a greater fall in the ascorbic acid content of the adrenals than did the resistant species. This effect was particularly pronounced with gophers. Whereas during the hibernation period injection of toxin had no effect on the ascorbic acid content of the adrenals and the spleen, and caused only a slight fall in its content in the lungs, and a slight rise in the liver, after the spring wakening the same dose of toxin caused a fall in the ascorbic acid content of all the organs examined, most of all in the adrenals.

The fall in the vitamin C content of the adrenals found following exposure to extraordinary stressor agents is regarded as an expression of enhanced functional activity of the adrenal cortex, in response to the stimulatory action of adrenocorticotrophic hormone [7, 8, 9]. The species differences found by us in the extent of glycogenolysis in the liver and in the fall in ascorbic acid concentration of the adrenals of animals given plague toxin are evidence that the response of the organisms to plague toxin is determined not only by the nature of the agent used, but also by the reactivity of the given species of animal.

The nature of these species differences in the extent of disturbance of carbohydrate and ascorbic acid metabolism following administration of plague toxin is obscure. It has been found in our laboratory that plague toxin causes intensification of the medullary function of the adrenals, modifying the reactivity of various systems of the organism to acetylcholine, histamine, and adrenalin, as also the reactivity of the carbohydrate metabolism of the liver in response to adrenalin, histamine, and insulin. It hence appears probable that the differences in degree of disturbance of carbohydrate metabolism in different species of animals suffering from the effects of plague toxin are related to species peculiarities in disturbance of neurohumoral regulation. It may also be supposed that an important part is played in the way in which carbohydrate metabolism is disturbed in plague intoxi-

cation by differences in the degree of inactivation of various enzyme systems participating in carbohydrate metabolism. The elucidation of these questions may be of interest both for the study of the pathology of plague and for the understanding of the mechanisms responsible for differences shown in the sensitivity of different species to plague toxin.

SUMMARY

The effect of plague toxin (fraction II of Pasteurella pestis No.137) on the liver glycogen and blood sugar contents, and on the ascorbic acid contents of the adrenals, spleen, liver, and lungs has been examined for five species of rodents, which differed in their sensitivity to the toxin; these were: white mice (highly sensitive), gerbils (Meriones meridianus nogaiorum Heptn.) and emuranchiks (Scirtopoda telum Licht.) (less sensitive), and guinea pigs and dwarf gophers (Citellus pygmaeus Pall.) (highly resistant). Intraperitoneal injection of toxin was followed within 30 minutes by a fall in liver glycogen content, a rise in blood sugar level, and a fall in the ascorbic acid content of the organs examined. The magnitude of the changes observed showed some correlation with the sensitivity of the given species to the toxin. Injection of toxin into hibernating dwarf gophers had no effect on their liver glycogen or blood sugar contents, or on the ascorbic acid contents of the adrenals and spleen, whereas in the spring, after termination of hibernation, these animals were as sensitive to the toxin as were white mice.

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MITOGENETIC RADIATION OF NEUROMUSCULAR SYSTEM AS A METHOD OF ANALYZING ITS MOLECULAR SUBSTRATE

PAPER IV. MANIFESTATION OF REGULATORY POWERS OF MOLECULAR SUBSTRATE OF MUSCLES IN VARIOUS FUNCTIONAL STATES

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In our previous papers [2, 3, 4] we developed the idea that an unbalanced state of order of the molecular substrate was a specific feature of the nervous system and muscles in the resting state.

The unbalanced state is maintained by the interaction of different – antibatic – processes produced by the spinal centers and muscles. In other words, the molecular substrate is constantly regulated by the system as a whole.

This gave rise to the question of whether regulative effects might be the basic feature of various functional states, which could then be regarded from a common viewpoint.

Hence, it was of interest to analyze the effects observed in frog muscle in experiments in vivo when the nerve was subjected alternately to subliminal and supraliminal stimulation.

METHOD OF EXPERIMENTS

The frogs (Rana temporaria or Rana ridibunda, mainly males) were pinned on a flat cork board. The gastrocnemius muscle was exposed and its tendon was cut and connected to the recording arm. A small portion of the central part of the sciatic nerve was also exposed and the electrodes were applied to it. The tetanic stimulations from an electric stimulator were characterized by the following parameters: frequency 15-20 cps, pulse duration 8-10 m sec, amplitude 0.1-2 v (the precise data are given in Table 1).

The area of the radiating surface of the muscle varied from 4-5 to 10-12 mm, depending on the aim of the experiments.

In the spectral experiments the muscle radiation was directed into the entrance slit of a spectrograph by reflection from a concave mirror. The radiation was detected by the usual biodetection method used in the laboratory [5, 6].

RESULTS OF EXPERIMENTS

The stimuli were applied in the following order: Supraliminal stimuli followed 20-30 sec later by subliminal stimulation for 5 to 10-12 sec (of the same or lower frequency), and after approximately the same time a second supraliminal stimulation of the same strength and frequency as the initial stimulation.

TABLE 1

Change in State of Muscle and Its Radiation Due to Subliminal Stimulation

Amplitude of stimulus (in v)		Muscle extension	Subsequent increase in muscle excitability	Muscle radiation (effect, %) for radiating surface of 10 mm², exposure 3 sec	Nos. of kymograms illustrating individual experiments
supraliminal stimulation	subliminal stimulation				
0,15	0,12	—	+	34	—
0,8	0,4	—	+	45	—
0,4	0,3	—	—	0	—
0,8	0,7	—	+	25	—
1,1	0,9	—	+	33	—
0,2	0,15	—	—	—8	—
0,8	0,5	+	+	40	6
1,8	1,5	—	+	35	—
0,2	0,15	—	—	—4	—
0,15	0,1	—	—	0	—
0,12	0,1	+	+	42	55
2	1,8	+	+	25	—
0,4	0,2	+	+	33	—
0,2	0,15	—	—	—5	—
0,2	0,12	—	+	50	66
0,8	0,5	—	—	—2	—
0,2	0,1	—	—	2	—
0,2	0,15	+	+	32	—
0,15	0,1	—	+	25	—
0,2	0,15	—	—	0	—
0,3	0,2	—	+	45	—
0,15	0,085	+	+	30	43
1,1	0,9	—	+	42	—

In 60-70% of the cases the second supraliminal stimulation resulted in a stronger muscular contraction than the initial stimulation, i.e. the excitability of the muscles was increased.

At the same time (in approximately 30% of the experiments) a slight extension of the muscle was observed during subliminal stimulation.•

In every case where an increase of excitability occurred the radiation intensity during subliminal stimulation increased considerably, irrespective of whether there was an evident extension or not.••

The results of the experiments are given in Table 1 and some of them are shown in the kymograms.

A comparison of the kymograms (see figure) shows that the important factor is the change of intensity of the stimulation, and not the frequency.

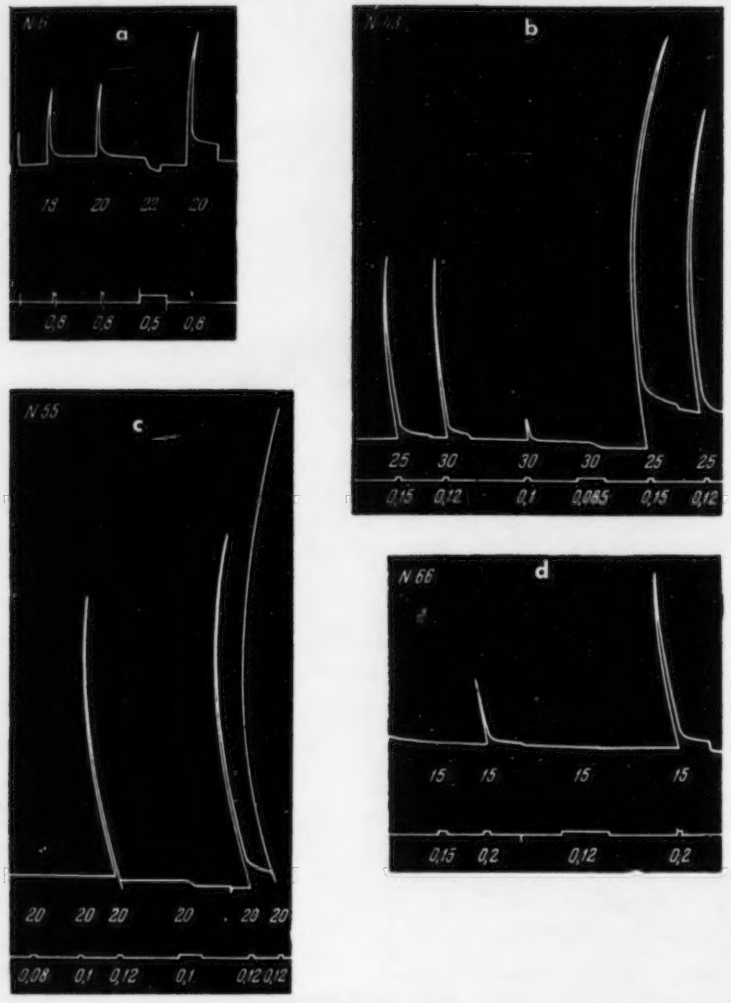
From previous laboratory data we knew that muscular contraction was also associated with a considerable intensification of radiation [5, 6]. But at the same time it seemed very probable that the states of the molecular substrate of the muscle during subliminal and supraliminal stimulation were different.

Hence, as criteria for assessing the state of the substrate we studied the changes in intensity of the muscle radiation during the action of stimuli lasting as long as 10-12 sec, and the presence of lines characteristic of free end-groups of peptides in the spectrum.

• Apparently the same effects resulting from weak stimulation were observed by Rauh [7]. In their phenomenology the described effects were also very similar to the picture of true pessimal inhibition discovered by I. A. Arshavskii and M. N. Kondrasheva [1].

•• The radiation intensity was determined, as usual, from its inverse dependence on the threshold exposure. As we know, for a resting muscle the threshold exposure for the same radiating surface is equal to 8 sec, i.e. the radiation of a resting muscle is about a third of that observed in these experiments [2, 3, 6].

As already noted, the increase and decrease in the number of free end-groups of peptides indicates not only corresponding changes in the dispersion of the substrate in the chemical sense of the term, but also changes in the weak intermolecular interactions responsible for the mutual orientation of the molecules [2]. In application to the unbalanced molecular substrate of the nervous system and muscles effects of such kind are involved.



Change in state of muscles during and after subliminal stimulation. Distinct extension of muscle during subliminal stimulation with subsequent increase in excitability (Nos. 6, 43, 55). Subsequent increase in excitability with scarcely noticeable extension (No. 66).

The effects discovered in muscle during subliminal and supraliminal stimulation were, in fact, different.

During subliminal stimulation a spectrum typical of peptide end-groups $R-NH_2$ and $R-OH$ was clearly displayed (Table 2).

During supraliminal stimulation, i.e. during muscular contraction, there was a rapid decrease in the intensity of these lines.

TABLE 3

Intensity of Muscle Radiation at Different Times After Start of Subliminal and Supraliminal Stimulations of Nerve

Subliminal stimulations		Supraliminal stimulations	
exposure	effect (in %)	exposure	effect (in %)
3 sec after start of stimulation	3 23 10 30 −3 40 0 40 10	3 sec after start of stimulation	0 −5 8 3 35
		3 sec in the period 3-7 sec after start of stimulation	12 −9 0
3 sec in the period 3-7 sec after start of stimulation	42 33 45 40 50	5 sec after start of stimulation	43 53 37 30 44 49
3 sec in period 7-11 sec after start of stimulation	35	5 sec in period 3-8 sec after start of stimulation	30 20
		5 sec in period 5-10 sec after start of stimulation	−10 3

If the state of the substrate of the neuromuscular system owes its character to the continuous interaction of elements contained in it, i.e. for each element "bound" in some degree to it, then the applied stimulations, by introducing even local changes in the substrate, must, as it were, increase the autonomy of the elements of the system, since they inevitably destroy or weaken the interaction. The direct consequence of this both for centers and muscles must be an intensification of the processes specific to them [2]; for muscles—intensification of their specific processes of a prepolymerization nature, which may lead to a decrease in free end-groups of peptides and a weakening of radiation; for centers—intensification of processes of a dispersive nature.

When stimulation ceases, the development of interacting processes again becomes possible. But their effectiveness cannot be the same for some period of time. Owing to the more balanced state of muscle substrate during contraction its energy level immediately after stimulation is lowered, i.e. central processes of a dispersive nature must predominate for a time.

But, proceeding from the idea of an unbalanced molecular order of the substrate, sustained by the interaction of antibatic processes, we must assume that the response to a predominance of dispersive processes will be an intensification of polymerizing process produced by the muscles. In other words, the active interaction of centers and muscles will be restored.

From our viewpoint this is a necessary condition for the return of the muscle to the resting state.

The increase in lability of the substrate, i.e. the greater excitability of the muscles, may be associated with these processes.

The suggested sequence of processes can be described as the manifestation of the regulative interactions of centers and muscles and, thus, the view that continuous regulation of the molecular substrate by the system as a whole is the basic feature of various functional manifestations, seems to us well-founded.

The last result corresponds with the data given below, which show that the total intensity of radiation of contracting muscle, the spectrum of which in the first few seconds contained many bands, gradually fell. On the other hand, the radiation intensity during subliminal stimulation even increased a little.

TABLE 2

Spectrum of Peptide End-Groups in Muscle Radiation During Subliminal Stimulation of Nerve

Functional group	Wavelength (in A)	Effect (in %)*
R—NH_2	2065—2070	41
R—NH_2	2260—2270	45
R—OH	1980—2000	35
R—OH	2400—2440	36

* Each figure is the mean from five experiments.

The radiating surface of the muscle in the experiments described below was reduced to 4-5 mm^2. In this case the stability of the positive effects was more clearly demonstrated (Table 3).

Thus, the data given in Table 3 show that the intensity of muscle radiation during subliminal stimulation exceeded the intensity during supraliminal stimulation and that the distribution of the intensity in time was different. During subliminal stimulation the intensity increased during the first 3-4 sec and then remained at the same level—the results were variable in the first few seconds and the reproducibility was good at subsequent times.

During supraliminal stimulation the level of radiation, comparatively high in the first 7-8 sec, subsequently fell.

In our examination of the obtained results we proceeded from the view that regulative rearrangements of the molecular substrate underlay the observed effects.

The most general definition of the effects observed during subliminal stimulation is that they have the nature of a possible response, and not of an obligate reaction, i.e. in contrast to the obligatoriness of muscular contraction under the action of a stimulus of corresponding intensity stimuli of lesser, but variable, intensities cause distinct changes in state in most cases, but can also fail to produce results.

We think that this distinctive type of phenomenology can be understood on the basis of the following viewpoint.

The dynamicity of the substrate of the nervous system and muscles, i.e. the continuous maintenance of an unbalanced state by the interaction of centers and periphery is clearly expressed, as we know, in the resting state. We assume that the unbalanced state is still continuously maintained during subliminal stimulation. But it is obvious that for this the substrate changes introduced by stimulation* must be compensated to a certain extent by the system itself. We regard the effects observed in the majority of cases as a manifestation of such compensation or, in other words, as the active manifestation of the regulative power of the substrate. These effects, of course, like any regulative processes, do not necessarily occur.

The mitogenetic data do not give direct evidence of this general view, but they certainly help to substantiate it.

A decrease in aggregability of the substrate (increase in free end-groups of peptides) lending to an increase in its mobility can only promote its dynamicity, i.e. the manifestation of regulative powers. A lesser degree of aggregability may also favor extension of the muscle and its subsequent increase of excitability.

At the same time, the increase in intensity of muscle radiation during subliminal stimulation (increasing with increasing stimulation), and which is presumably due to the more frequent occurrence of disturbances and restorations of the molecular order of the substrate, is quite naturally regarded as a manifestation of its increased dynamicity.

In the case of supraliminal stimulation the very fact of the obligatoriness of the contraction response, the gradual weakening of radiation and, in particular, the rapid disappearance of the spectrum of peptide end-groups, show that under continuing stimulation the muscle substrate passes into a new, i.e. more balanced, state. After stimulation, however, the muscle returns to the initial state, and we will attempt to examine this phase from the standpoint of the antibatic nature of the interacting processes produced by the centers and muscles.

* Particularly effective, presumably, during the short interval after supraliminal stimulation.

SUMMARY

This work deals with the reaction of M. gastrocnemius to the subliminal stimulation of the sciatic nerve in experiments on frogs (in vivo). In the majority of the cases there occurs a slight extension of the muscle with a subsequent rise of its excitability, connected with a considerable intensification of mitogenetic radiation. These phenomena are the manifestation attending the regulatory reconstructions of the molecular muscular substrate caused by an active interaction of the centers and muscles. The changes in the substrate connected with the supraliminal stimulation, especially during the return of the muscle from the state of contraction into the state of rest, are regarded from the same point of view.

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ADJUVANT EFFECT OF 5-PHENYL-3-METHYLPYRAZOLE (PHEMERAZOLE) ON THE ACTION OF DIETHYLSTILBESTROL ON THE MAMMARY GLANDS OF RABBITS

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In our earlier researches we established some of the pharmacological properties of 5-phenyl-3-methylpyrazole (Phemerazole) [1]. Phemerazole has a tranquilizing action on the central nervous system of experimental animals, lowers their body temperature, counteracts the stimulatory effect of a number of drugs, and enhances the action of soporific drugs [2, 3]. Because of these properties, Phemerazole could be classed as a sedative drug. Such drugs powerfully affect the central nervous system, and also act on certain of the endocrine glands; this latter effect is manifested as enhancement or depression of their function, with corresponding effects on the target organs. An example of this kind of effect is afforded by clinical reports of occurrence of lactation in women, and of mammary enlargement, with formation of secretion, in men, following prolonged treatment with chlorpromazole or reserpine [4, 6, 8]. H. Tuchmann-Duplessis and L. Mercier-Parot [9] have reported mammary enlargement in both male and female rats following 8-15 daily injections of 250 mg/kg of reserpine. The lobules were enlarged, and the ducts were filled with secretion. C. H. Sawyer [7] and J. Meites [5] administered estrogens to rabbits, at dosage levels such as did not have any perceptible effect on their mammary glands. Considerable enlargement of the mammary glands was observed when reserpine was injected intravenously (a single dose of 1 mg/kg), following two preliminary injections of estrogen. The different response of the rabbits to estrogen treatment was evident by the seventh day after reserpine administration. The majority of workers believe that mammary enlargement following chlorpromazine or reserpine administration may be ascribed to increased secretion of lactogenic hormone by the anterior hypophysis, with suppression of luteal function. The way in which these drugs act has not yet been fully elucidated. C. H. Sawyer and J. Meites found that the mammary effects of the drugs appeared sooner, and were more marked, when supplementary doses of estrogens were given.

EXPERIMENTAL METHODS

We examined the effect of Phemerazole on the mammary glands of the rabbit in the same way as did C. H. Sawyer, with supplementary administration of estrogens. The experiments were performed on female rabbits, weighing 2-2.8 kg. The estrogen used was diethylstilbestrol, which was injected subcutaneously as a 2.5% suspension in aqueous alcohol, at a dosage level of 0.1-0.2 mg/kg. Phemerazole hydrochloride was fed as a suspension in water, at dosage levels of 25 and 100 mg/kg. The rabbits were killed 7-8 days after the last injection of diethylstilbestrol.

RESULTS OF EXPERIMENTS

Two daily injections of 0.1 mg/kg of diethylstilbestrol did not give any mammary enlargement or secretion (see table), showing that this dosage level was too low for our purposes. Raising the daily dose to 0.2 mg/kg gave

Results of Combined Administration of Phemerazole and Diethylstilbestrol

Single peroral dose of Phemerazole (in mg/kg)	Time interval between administration of Phemerazole and the first injection of diethylstilbestrol	Dose of diethylstilbestrol (in mg/kg) and the number of such subcutaneous injections given	Number of rabbits per group	Results of examination of the mammary glands of rabbits 7-8 days after the last injection of diethylstilbestrol. Number of rabbits in which we observed:			
				absence of mammary enlargement	slight mammary enlargement	mammary enlargement, with appearance of secretion	considerable mammary enlargement, with plentiful secretion
100	7 days	0.1×2	4	3	1	–	–
Control	–	0.1×2	4	4	–	–	–
100	7 days	0.2×2	4	–	2	2	–
Control	–	0.2×2	4	1	3	–	–
100	2 hours	0.2×10	4	–	1	1	2
25	2 hours	0.2×10	4	–	1	3	–
Control	–	0.2×10	4	1	2	1	–
100	2 hours	0.2×15	4	–	1	–	3
Control	–	0.2×15	4	–	3	–	1

a more or less definite effect on the mammary glands, depending on the number of doses given. Two subcutaneous injections of 0.2 mg/kg gave slight enlargement of the mammary glands in 3 out of 4 animals; the remaining rabbit gave no response. Enlargement of the mammary glands of all four of a group of rabbits was observed when a subcutaneous injection of 100 mg/kg of Phemerazole hydrochloride had been given seven days before the first diethylstilbestrol injection, and secretion was seen in the lobules of two of them.

Fig. 1. Mammary gland of a control rabbit following 10 subcutaneous injections of diethylstilbestrol (0.2 mg/kg per dose). Slight enlargement of lactiferous ducts. Magnification: oc. 8 ×, ob. 10 ×.

Fig. 2. Mammary gland of a rabbit given a preliminary subcutaneous injection of 100 mg/kg of Phemerazole hydrochloride, followed by 10 injections of diethylstilbestrol (0.2 mg/kg per dose). Hyperplasia of the mammary alveoli, and distension of the ducts. Magnification: oc. 8 ×, ob. 10 ×.

In two series of experiments (20 rabbits) subcutaneous injection of 0.2 mg/kg of diethylstilbestrol was begun two hours before the single injection of Phemerazole, and was continued for 10 and 15 days. The control group of rabbits was given diethylstilbestrol alone. The animals were killed 7 days after the last injection of diethylstilbestrol. The mammary glands of the rabbits which had been given both Phemerazole and estrogen were considerably enlarged, and displayed enhanced secretory activity. The lactiferous duct system was highly developed, and the ducts were distended by their large content of watery, sometimes turbid, secretion; numerous alveoli were visible without the aid of a microscope. The peripheral regions of the mammary glands were markedly hyperemic (Figs. 1 and 2).

These changes in the mammary glands were always more pronounced in the animals which had been given Phemerazole than in those receiving diethylstilbestrol alone.

Histological examination of the mammary glands of the rabbits showed hypertrophy and hyperplasia of the lactiferous and alveolar ducts, with considerable distension of their lumina by a protein-containing fluid, in which sporadic clumps of desquamated epithelial cells could be seen. The ducts, and many of the alveoli, were lined with stratified squamous cells, or, less frequently, with cuboidal or columnar epithelial cells. The stroma was sporadically hyperemic, and small clumps of round-cell elements could be seen around some of the alveoli. The mammary glands of the control group of rabbits, which had been given multiple injections of stilbestrol alone, showed slight enlargement, with inconsiderable dilatation of the lactiferous ducts, which in some cases contained small amounts of serous fluid.

Our experiments showed that the action of diethylstilbestrol on the mammary glands of rabbits is intensified by preliminary administration of Phemerazole. In this respect, Phemerazole resembles the sedative drugs chlorpromazine and reserpine.

SUMMARY

The action of diethylstilbestrol on rabbit mammary glands is intensified by administration of Phemerazole (5-phenyl-3-methylpyrazole). Enlargement of the mammary glands is seen, with considerable dilatation of ducts and alveoli, which become filled with secretion. Histological examination showed hypertrophy and hyperplasia of glandular tissue, with considerable dilatation of the lumina, which were filled with protein-containing secretion in which desquamated epithelial cells could be seen.

The control group of rabbits, which received diethylstilbestrol alone, showed only slight dilatation of the lactiferous ducts.

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STUDY OF AUTOSENSITIZATION IN RADIATION SICKNESS BY HOIGNÉ'S METHOD

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(Presented by Active Member AMN SSSR N. N. Zhukov-Berezhnikov)
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It is well known that autosensitization develops in many diseases in which cell structures are destroyed and breakdown products are absorbed. There are experimental data indicating the important role of this effect in the pathogenesis of radiation sickness, also [3]. For instance, local hyperergic reactions to tissue breakdown products have been obtained [4, 5], and the appearance of auto-antibodies to denatured protein of tissues and cytolysins has been established [1, 2].

In the search for a method capable of revealing the ability of sera of irradiated animals to react with auto-allergens in experiments in vitro we decided to use Hoigné's method, an account of which was published in 1955 [7-10]. This method was proposed for the demonstration of allergy in humans towards various medicines. The essential feature of this method is the employment of a highly sensitive photoelectric nephelometer to determine the turbidity produced by the reaction occurring when the sensitizer-allergen is added in optimal concentration to the plasma or serum of the patient's blood. Since this concentration is unknown, increasing amounts of antigen are added successively to the same sample of serum. In samples taken from normal people there is a steady decrease in the optical density of the medium.

In the blood plasma of sensitized persons combination with antibodies occurs at a particular range of dilution of the allergen, and this is manifested in a stoppage in the fall of the optical density of the medium (plateau on the curve) or even in an increase in density. When more allergen is added the optimal relationship with the antibody is upset and the reduction in turbidity of the mixture begins again.

We attempted to employ Hoigné's method (which some authors [6, 11] estimate as highly sensitive) for a study of the nature of the interaction of the plasma of normal and irradiated animals with aqueous extracts of intestine, liver, spleen, or bone marrow of the same organism.

METHOD AND RESULTS

To 1.7 ml of freshly prepared citrate blood plasma we added a succession of 0.1 ml batches of centrifuged aqueous extract of the organ in dilution 1:5120 to 1:80. After each addition we stirred the mixture in the glass with a glass rod. Two minutes later we measured the optical density of the medium on the home-produced photoelectric nephelometer (FEKN-57), and then added the next batch of allergen.

Experiments conducted on normal rabbits [11], guinea pigs and rats [7] showed that the blood plasma of these animals did not react with extracts of internal organs. In every case a steady decrease in the optical density of the mixture was noted. But after the animals had been exposed to lethal X-ray doses* we observed the appearance of a plateau on the curves and a temporary increase in the optical density of the plasma. This was re-

* The guinea pigs (300 r) and rats (600 r) were irradiated on the RUM-3 X-ray apparatus at voltage 180 kv, current 15 ma, focal distance 50 cm for guinea pigs and 40 cm for rats, dose rate 19-20 r/min, filters 0.5 Cu+1 mm Al. The rabbits were exposed to a dose of 800 r on the RU-12 apparatus at the same voltage and with the same filters, focal distance 60 cm, current 14 ma, dose rate 29.8 r/min, with three tubes operating.

garded as a positive reaction. It occurred most often in the case of interaction with liver extract. Five out of 14 rabbits killed at different times after exposure to 800 r showed a positive reaction on the 3rd or 9th day. The results of one of the tests are shown graphically in Fig. 1.

The tests were positive (on 3rd-5th day) in eight out of 16 guinea pigs exposed to 300 r. The positive reactions occurred most often in the experiment involving the addition of liver extract, less often with bone marrow and spleen, and occasionally with intestine.

In an experiment on eight rats exposed to 600 r we used an extract of a mixture of equal quantities of all the organs mentioned. We observed positive reactions when extracts from normal nonirradiated animals were added to the plasma of irradiated rats.

The animals had to be killed for these experiments, however, and this ruled out the possibility of a study of the dynamics of the investigated effects in the same organism. Hence, we devised a method for obtaining solutions of autoantigens for the repeated conduction of the tests during the life of the animal. As an allergen we used a lysate of formed blood elements (mainly erythrocytes), which are known to possess antigens common to other cells of the organism. To obtain this allergen we took blood from the fasting animal, mixed it with a sodium citrate solution in the usual proportions, centrifuged the mixture, and washed the deposit twice with physiological saline. After this we added distilled water till the volume was equal to the initial volume of blood and again centrifuged the mixture. The clear colored upper layer was used in dilutions 1:15 360 to 1:30 in the experiments.

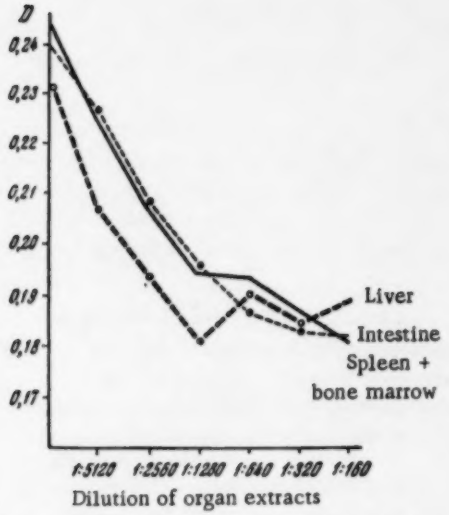

Fig. 1. Curves showing decrease in optical density of plasma on successive addition of increasing concentrations of extracts of organs of same rabbit killed on third day after exposure to X-rays. Distinct positive reaction with liver extract, and positive reaction with extract of mixture of bone marrow and spleen.

Experiments conducted on 12 rabbits and 19 dogs showed that in normal animals the successive addition of batches of erythrocyte lysate caused a progressive reduction in the optical density of the mixture. Four rabbits were subjected to homosensitization (intravenous injection of 5 ml of 20% extract of liver and intestine in equal proportions) and eight were exposed to a dose of 800-1000 r. Both types of treatment resulted in a change in the curves in a number of cases. A plateau appeared on the 3rd-5th day after treatment, thus indicating a reaction between the plasma and the organ extracts.

The main group of experiments by the method employing a lysate of autoerythrocytes was conducted on 19 dogs weighing 9-12 kg, which were exposed to a dose of 300 r, i.e. a lethal dose, of Co^{60} γ-rays* on the EGO-2 apparatus in uniform-field conditions at dose rate 350 r/min. Eight rabbits formed the control group, and 11 were treated with preparations of antibiotics and vitamins. In the experimental group six dogs survived and five died. All the animals showed the clinical picture of acute radiation sickness. The tests were made before exposure and on the 3rd, 10th, 20th, 30th, and 45th day after exposure. On the 3rd day we observed considerable changes in the properties of the plasma of irradiated dogs. There was a considerable reduction in the optical density of the plasma itself. This reduction, as Fig. 2 shows, persisted throughout the period of radiation sickness, both in the treated and in the control animals. Moreover, the blood plasma became capable of interacting with the lysate of its own erythrocytes. This was revealed by the appearance of a plateau on the curve, or even by an increase in optical density (Fig. 3). This last type of reaction had an unfavorable prognostic significance, since all the dogs in which the blood plasma revealed such a reaction died. Positive reactions occurred, both in the control and in the experimental group of animals (see Table). But in the experimental dogs they appeared 7-10 days later and their total number (57.5%) was less than in the untreated animals (84.2%). We noted

*Exposure to 300 r of Co^{60} γ-rays on the EGO-2 apparatus is equivalent in biological effect to exposure to a 600 r dose of X-rays.

Number of Positive and Negative Reactions of Dog Blood Plasma with Lysate of Its Own Erythrocytes

Group of dogs	Reaction	Before exposure	Day of investigation after exposure					Total number of positive reactions during first 20 days of radiation sickness	
			3rd	10th	20th	30th	45th	in absolute figures	in per cent
Control	Negative	8	1	1	1	–	–	3	15.8
	Positive	0	7	6	3	–	–	16	84.2
	Total . .	8	8	7	4	Dogs	died	19	–
Experimental (treated animals)	Negative	11	6	4	2	2	3	17	42.5
	Positive	0	5	6	5	4	3	23	57.5
	Total . .	11	11	10	7	6	6	40	–

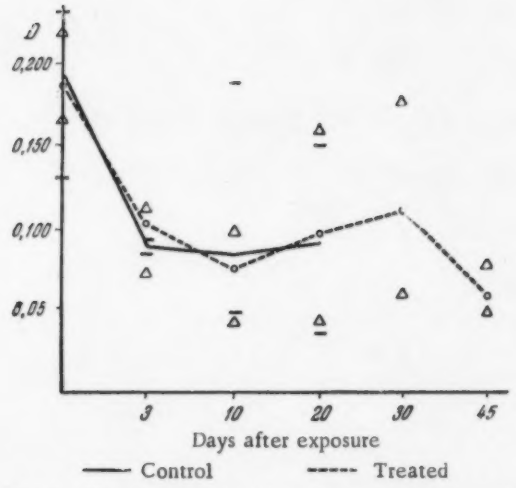

Fig. 2. Fluctuations of optical density of plasma of treated and control animals during acute radiation sickness.

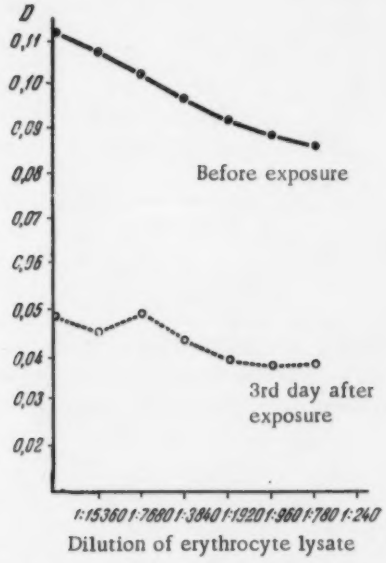

Fig. 3. Positive reaction of blood plasma of irradiated dog with lysate of its own erythrocytes on 3rd day after exposure.

a restoration of the normal form of the curve in the case of the surviving animals, although in some of them positive reactions still occurred on the 45th day.

Thus, in the early period after exposure to a lethal dose of X-rays or Co^{60} γ-rays Hoigné's method can reveal the immunological properties of the blood plasma in its interaction with a lysate of its own erythrocytes. We believe that the manifested ability of the plasma to react with cell breakdown products indicates the existence of a state of autosensitization: The addition of lysates of erythrocytes of animals of another species, foreign serum, or physiological saline to the plasma of irradiated animals caused a decrease in optical density of the mixture, i.e. a negative reaction. The diagnostic and prognostic significance of this reaction in radiation sickness produced by the action of various doses and kinds of ionizing radiation requires further study.

The immunological nature of the investigated reaction was confirmed by experiments with the plasma of rabbits immunized by foreign protein (of guinea pig). The successive addition of heterologous antigen resulted

in a steady decrease in optical density, while the addition of homologous antigen caused an increase in turbidity after a certain concentration of antigen was reached. We suggest that the speed with which the increase in optical density is manifested can be used to assess the avidity of the serum, since in this experiment we used sera with an equal titer of precipitins, but the times of appearance of the positive reaction were different. Thus, Hoigné's method can also be used in serum production. We should note that the intensity of the reaction to foreign antigen is much higher than to autoantigens, since the initiated increase in optical density becomes steadily greater and does not give way to a decrease, as is the case in interaction with tissue products.

SUMMARY

A method for preparing autoallergen solutions was developed (the liver, spleen, bone marrow, and intestine water extracts and lysates of erythrocytes) to stage the reaction according to the Hoigné method with the blood plasma of irradiated animals. As established, when tissue allergen solutions were added in increasing concentrations to the blood plasma of nonirradiated animals a constant and uninterrupted reduction of the optic density, i.e. negative reaction was seen. Beginning from the 3rd day after irradiation with lethal doses of γ-rays (Co^{60}) or X-rays, blood plasma acquires the ability to become bound with tissue antigens; this is manifested in the delayed optic density reduction of the medium, or even in its increase, which, according to Hoigné is evaluated as a positive reaction. The authors hold that the data obtained point to an early development of autosensitization in the irradiated organism.

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MECHANISM OF INHIBITION OF ANAPHYLACTIC REACTION BY NARCOSIS*

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About 60 years have passed since Richet's classical experiments (1902), which led to the formulation of the main views on anaphylaxis. These were concerned more with the phenomenological aspect of the problem. Yet there is still no satisfactory explanation of the mechanisms of anaphylaxis. The vast literature on this question indicates the great difficulties encountered in its solution.

One of the lines of research on the mechanisms of anaphylaxis is an investigation of the role of the nervous system in the pathogenesis of the anaphylactic reaction. This problem has been investigated by several experimental methods—by pharmacological alteration of the functional state of the central nervous system, mainly by narcosis; by extirpation of various parts of the central nervous system, by intradural injection of the anaphylactogen, and by conditioned-reflex reproduction of the anaphylactic reaction.

The greatest number of experiments, however, have been devoted to a study of the effect of narcosis on anaphylactic shock. A. M. Bezredka [4] was the first to show that narcosis inhibits anaphylactic shock. These initial data have been confirmed several times, and in recent years have been supplemented by a study of the effect of narcosis on other allergic reactions—the Arthus-Sakharov phenomenon, the Schwartzmann phenomenon, tubercular allergy, and brucellar allergy [3, 5, 10, 11].

But narcotic substances are known to act not only on the nervous system, but also on other organs and tissues of the organism, and to produce changes in metabolism, permeability, and several other tissue changes which have some bearing on allergic reactions [8]. Hence, several immunologists believe that the mechanism of the observed inhibition of the allergic process is obscure and that the role of the nervous system in this phenomenon has not been proved, since narcosis may have a toxic effect on the smooth musculature.

The aim of this work was to investigate the mechanism of the inhibition of the allergic reaction by narcotics.

METHOD

To resolve this question we combined the method of pharmacological alteration of the functional state of the central nervous system with the classical Dale-Schultz method of investigating the tissue sensitivity of an isolated organ. We called this combined method of investigation the cross-checking method. It consists in a determination of the role of the narcotic state in the course of the anaphylactic reaction in the intact organism and a subsequent study of the tissue sensitivity to the anaphylactogen in an isolated organ—the uterus of the same animal—by means of the Dale-Schultz method [12] (A. T. Kravchenko and N. V. Galanova [1948]).

* I consider it my duty to express my sincere thanks to Corresponding Member AN SSSR Kh. S. Koshtoyants for kindly providing the facilities for the conduction of this work.

TABLE 1

Comparative Study of Sensitivity of Isolated Uterine Horn to Horse Serum, Histamine, and Acetylcholine After Injection of Shocking Dose of Protein Into Narcotized Animals

No. of experiments	Horse serum			Histamine			Acetylcholine		
	dilution	exposure (in min)	reaction	dilution	exposure (in min)	reaction	dilution	exposure (in min)	reaction
3	$1:10^{-5}$	1—5	—	$1:10^{-8}$	1—5	— (1+)	$1:10^{-7}$	1—5	— (1+)
10	$1:10^{-4}$	1—5	—	$1:10^{-6}$	1—2	+	$1:10^{-5}$	1—2	+
5	$1:10^{-3}$	1—5	— (1+)	$1:10^{-7}$	1—5	— (2+)	$1:10^{-6}$	1—2	+
2	$1:10^{-2}$	1—5	— (1+)	$1:10^{-5}$	1—2	+	$1:10^{-4}$	1—2	+

Symbols: Absence of muscular contraction; + presence of muscular contraction. Figure in brackets denotes number of experiments in which a positive reaction was obtained.

The experiments were performed with 50 guinea pigs, weighing 300-420 g, which were divided into five groups (two experimental and three control, ten animals in each). All the animals were sensitized by a single subcutaneous injection of 0.1 ml of horse serum and 18-22 days later were taken for the experiment.

Narcosis was produced by the intraperitoneal injection of urethan, 1 g per kg of body weight, and lasted for 2-7 hr. The animals of the first experimental group were given a shocking dose (0.4 to 0.8 ml) of horse serum, intraperitoneally or subcutaneously, $1\frac{1}{2}$-$2\frac{1}{2}$ hr after the onset of deep narcosis.

The same dose of horse serum was injected into the ten pigs of the first control group. All the control animals reacted to the injection of the shocking dose of protein by severe and lethal shock.

In the animals of the first experimental group (first series of experiments) the shock was considerably weakened or completely inhibited by narcosis. After 24-36 hr we investigated the specific tissue sensitivity to horse serum on the isolated uterus of these animals in the experiment in vitro.

In the second experimental group (second series of experiments) the sensitized animals were decapitated $1\frac{1}{2}$-2 hr after the onset of narcosis and the reaction to specific protein was investigated on the isolated uterus in the experiment in vitro.

In only two out of the ten narcotized animals of the second control group did we observe a pronounced anaphylactic reaction after the injection of the shocking dose of protein. In the other animals the shock was greatly weakened or completely suppressed.

The experiments in vitro were carried out in the following way. The two uterine horns were separated by the usual method and put into the dishes (capacity 50 ml) containing Ringer-Locke solution at 37.5° on the Dale-Schultz apparatus. Oxygen was passed through the Ringer-Locke solution (50-60 bubbles per minute). The contractions of the smooth muscle of the isolated uterus were recorded on a kymograph by light scribes. After the uterine horns were placed in the dishes they were kept there for 15-35 min to acquire their initial tonus. The scribes were brought against the smoked tape immediately before the start of the experiment. Parallel records of the responses of the two horns were made. Normal sera in the following concentrations were used: horse serum $-1 \cdot 10^{-5}$, $1 \cdot 10^{-4}$, and $1 \cdot 10^{-3}$, nonspecific ram serum $-1 \cdot 10^{-4}$ and $1 \cdot 10^{-3}$. In addition, nerve mediators were used $-$ acetylcholine in concentrations $1 \cdot 10^{-7}$, $1 \cdot 10^{-6}$, $1 \cdot 10^{-5}$, and $1 \cdot 10^{-4}$, and histamine in concentrations $1 \cdot 10^{-8}$, $1 \cdot 10^{-7}$, $1 \cdot 10^{-6}$, and $1 \cdot 10^{-5}$. These concentrations of substances were final, i.e. their dilution by the liquid in the dish was taken into account.

The duration of action of each stimulus was decided by the aim of the experiment. The preparations were washed for 5 to 25 min after the action of the stimuli.

TABLE 2

Comparative Study of Sensitivity of Isolated Uterine Horn to Horse Serum, Histamine, and Acetylcholine 1½-3 Hr After Onset of Narcosis in Animals

No. of experiments	Horse serum			Histamine			Acetylcholine		
	dilution	exposure (in min)	reaction	dilution	exposure (in min)	reaction	dilution	exposure (in min)	reaction
10	$1:10^{-4}$	1—2	+	$1:10^{-6}$	1	+	$1:10^{-6}$	1	+
5	$1:10^{-3}$	1	+	$1:10^{-7}$	1	+	$1:10^{-7}$	1—2	+
5	$1:10^{-2}$	1	+	$1:10^{-8}$	1—2	+ (2—)	$1:10^{-5}$	1	+

Note. Symbols as in Table 1.

RESULTS OF EXPERIMENTS

In proceeding to the discussion of the experimental results we must mention that the threshold doses, determined in special experiments, were as follows: horse serum $1 \cdot 10^{-5}-1 \cdot 10^{-4}$, histamine $1 \cdot 10^{-8}-1 \cdot 10^{-7}$, acetylcholine $1 \cdot 10^{-7}-1 \cdot 10^{-6}$.

The threshold doses depended on the individuality of the animal and the time spent by the isolated preparation in the experiment.

Table 1 shows that animals under narcosis became desensitized after the injection of a shocking dose of protein, despite the absence of external symptoms of anaphylactic shock. Yet, it is quite obvious that combination of the antigen and antibody took place. The evidence for this was the onset of desensitization in all the experimental animals, and the isolated uterine preparation did not respond by muscular contraction to known supraliminal doses of protein.

As an illustration of this we show the kymograph trace of the responses of the uterine horn of experimental pig No. 7 (Fig. 1).

In this connection, it is important to recall that the onset of a state of antianaphylaxis after the injection of a shocking dose of protein into narcotized animals was first noted by A. M. Bezredka [1907], and then by A. T. Kravchenko and N. V. Galanova [9].

Yet, such an experimental procedure did not enable us to draw a definite conclusion about the mechanisms of the inhibition of anaphylaxis by narcosis, or to indicate its nature, nervous or tissue, since it is possible that narcosis, by altering the metabolic processes of the organ tissues, lowers their general reactivity. Indeed, for removal of this objection we used another two stimuli, histamine and acetylcholine, in the experiments, but this was indirect information. The experiments showed that tissue sensitivity to histamine and acetylcholine persisted in the desensitized animals. There was only a slight rise in the threshold of response. On the basis of these results, we can postulate different biochemical pathways for the action of the protein, on one hand, and histamine and acetylcholine, on the other.

For direct experimental evidence of the nervous nature of the inhibition of anaphylaxis by narcosis a second series of experiments was undertaken. The sensitized animals were narcotized and then decapitated 1½ hr after the onset of deep narcosis. Both uterine horns were used in the experiment in vitro, and the presence of sensitivity to protein, histamine, and acetylcholine was investigated. The results obtained are given in Table 2.

Table 2 shows that the tissues of a narcotized animal did not lose their sensitivity to a specific protein stimulus. The threshold of response of the isolated preparation to histamine and acetylcholine remained the same or was even a little lower. As an illustration of this fact we show a kymograph trace of the functional activity of the muscle in response to the action of specific protein for experimental guinea pig No. 15 (Fig. 2).

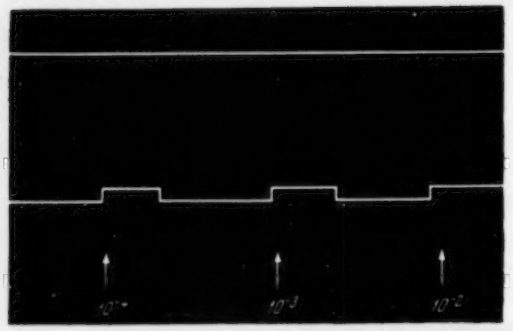

Fig. 1. Onset of desensitization in animal after inhibition of anaphylactic reaction by narcosis. Meaning of curves (top to bottom): trace of functional activity of uterine horn in vitro; stimulation marks. Vertical arrows denote moment of injection of horse serum. Serum concentration is indicated.

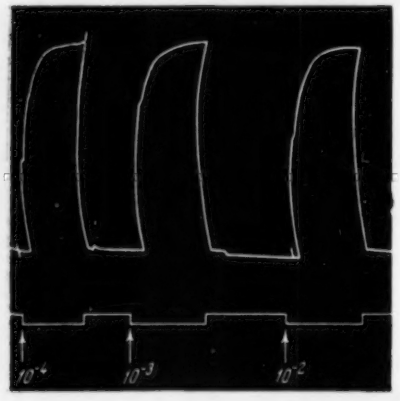

Fig. 2. Persistence of tissue sensitivity to specific protein 1½-3 hr after onset of narcosis. Meanings as in Fig. 1.

To verify the specificity of the reaction of the animal tissue to serum antigen we investigated the reaction to ram serum in dilutions $1 \cdot 10^{-4}$ and $1 \cdot 10^{-3}$ in ten experiments with five guinea pigs. The experiments showed that the organ taken from a narcotized animal did not contract in response to the action of the nonspecific ram serum.

The effect of narcosis itself on the reaction of an isolated preparation of a nonsensitized animal was also tested in ten experiments with five guinea pigs. There was no specific reaction to horse serum in these conditions—the organ did not contract.

An examination of the results of these experiments suggests that in such a multistructural reaction as the anaphylactic reaction there are two interconnected and interdependent phases: an immunochemical (or specific) phase, and a physiological (or nonspecific) phase.

The underlying feature of the immunochemical, or specific, phase is the immunological mechanism proper, the antigen-antibody reaction. The physiological, or nonspecific, phase of the anaphylactic reaction involves many systems and organs of the organism, primarily the smooth musculature, the level of functional activity of which can be altered by a great variety of factors, including antigenic factors, and this accounts for the nonspecificity of this phase of anaphylaxis.

The results of the two series of experiments, particularly the second series, and of the various control experiments have shown that the physiological phase of the anaphylactic reaction is blocked by interference with the functional state of the central nervous system, by the resultant inhibitory state, and not by poisoning of the tissues of the internal organs. From these experiments we can subscribe to M. Bezredka's opinion that the inhibition of anaphylaxis by narcosis is of a nervous nature, and also to the idea of the leading role of the central nervous system in the anaphylactic reaction [1, 2, 6] or, more precisely, in its physiological phase.

At the same time our experiments, and also the published information [4, 9, 13], require that the part played by nervous mechanisms in the course of such a complex, generalized reaction as the anaphylactic reaction must be defined and demarcated. Such a problem has been made possible by the differentiated approach to the clarification of the mechanisms of induction and development of the anaphylactic reaction and its separation into two phases—the immunochemical and physiological. From our experimental data, and also the data in the literature, we can conclude that the employed methods of investigation do not demonstrate a leading role for the nervous system in the realization of the immunochemical phase, since the combination of antibody with antigen (cause of desensitization) occurs even when the nervous apparatus is excluded. Here, however, we must mention the existence of trophic nervous effects, which can determine the degree of reaction between tissue substrate and antigenic stimulus and thus alter the intensity of the anaphylactic reaction, as some of our experiments indicate.

The decisive influence of the neurodynamics of the cortical-subcortical system is clearly demonstrated in the occurrence of the physiological phase of the anaphylactic reaction. Alteration of the established intercentral relations by the mechanisms of the cortical dynamic stereotype and induction relations [7] can lead to a pronounced weakening or complete inhibition of the physiological phase of the anaphylactic reaction.

SUMMARY

The mechanisms involved in the arrest of anaphylactic reaction by anesthesia were analyzed. As demonstrated, inhibition of anaphylactic reaction occurs at the expense of the changes in tissue sensitivity resulting not from the tissue poisoning by anesthetics, but from altered functional condition of the central nervous system. A suggestion is made that in such a complicated polystructural reaction as the anaphylaxis, there are two interconnected and interrelated phases: immunochemical and physiological or specific and nonspecific. The author's own data and that derived from the literature show that realization of the specific phase of anaphylactic reaction may be effected without the nervous system playing a leading part in it, whereas the nonspecific phase of this reaction occurs with the participation of the neuro-reflex control, as a decisive factor.

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THE ANTIGENIC PROPERTIES OF SOME FRACTIONS OF EHRLICH'S ADENOCARCINOMA OF MICE

COMMUNICATION II. THE STUDY OF THE ANTIGENIC PROPERTIES OF TUMOR FRACTIONS BY MEANS OF THE PRECIPITATION REACTION IN JELLY AND VACCINATION EXPERIMENTS IN MICE

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Original article submitted July 21, 1960

In the previous communication [2] we demonstrated the presence of specific and of species-specific antigens in a saline extract and in the globulin fractions of Ehrlich's adenocarcinoma.

The object of the present investigation was to make a detailed study of the antigenic structure of Ehrlich's adenocarcinoma and of its globulin fractions by means of the precipitation reaction in jelly, and to compare the vaccinating activity of the tumor fractions by vaccination experiments in mice.

EXPERIMENTAL METHOD

The precipitation reaction in jelly was carried out by Ouchterlony's method [13]. In a series of experiments a micromodification of the method was used: the diameter of the matrices forming the wells was reduced to 4 mm, and the distance between the wells containing antigen and antiserum was 5 mm. Lines appeared after 18 hours. One Petri dish could be used for 6-8 experiments.

The results of the reaction were recorded photographically by means of a "Zenith" camera, with oblique illumination from beneath provided by a special apparatus constructed by G. I. Avdeev. Some experiments were recorded on an MF-2 microphotometer by the original method. Use of the microphotometer enables a quantitative analysis of the fine and poorly differentiated precipitation lines to be made.

To produce antisera, rabbits were immunized intravenously with a suspension of washed ascites cells in accordance with a four-week cycle program.

Fine differentiation of the antigens in the reaction was obtained by using Björklund's method of specific inhibition of precipitation [9]. On the night before the experiment antigen was poured into all the wells. The jelly was saturated to excess with antigen, and no precipitation lines were formed to this antigen.

The vaccination experiments were carried out on mongrel white mice. In view of the considerable interest in the question of postoperative immunity, developing after the removal of a tumor [10, 12], in each series of experiments we immunized one group of mice by implantation of the tumor in the tail area by Andervont's

method [8] in order to compare the efficacy of chemical vaccination with that of vaccination with the virulent tumor. A suspension of ascites cells (0.1-0.2 ml) was injected from a tuberculin syringe beneath the skin of the tail, at the junction of the lower and middle thirds. After 9-14 days, when the tumor had reached a certain size, the tail was amputated. From 19 to 28 days after amputation the infecting dose of the tumor was injected subcutaneously into mice in which no recurrence was observed, and simultaneously into the mice of the other experimental groups.

The other groups of animals were immunized with a saline extract of the tumor, with globulin I and globulin II of the tumor (obtained by salting out with 46% and 50% saturated ammonium sulfate respectively), and with globulin from mouse liver and spleen. The dose of all the antigens was estimated in terms of dry weight of the lyophilized material. Each mouse received a single subcutaneous injection of 2 mg of antigen in the left side. From 11 to 12 days after vaccination an infecting dose of 0.2 ml of ascites fluid in a 1:4 dilution was injected beneath the skin of the animal's right side. The control group of unimmunized mice received injections of the infecting dose of tumor at the same time.

On the 16th day after inoculation all the animals were sacrificed and the tumors weighed. The experimental results were treated statistically: the weight of the tumor by the Fisher-Student method, the proportion of successful implantations of the tumor by the formula of the "fourfold" table [7]. The mean weight of the tumor was calculated only for those animals in which the tumor was successfully implanted.

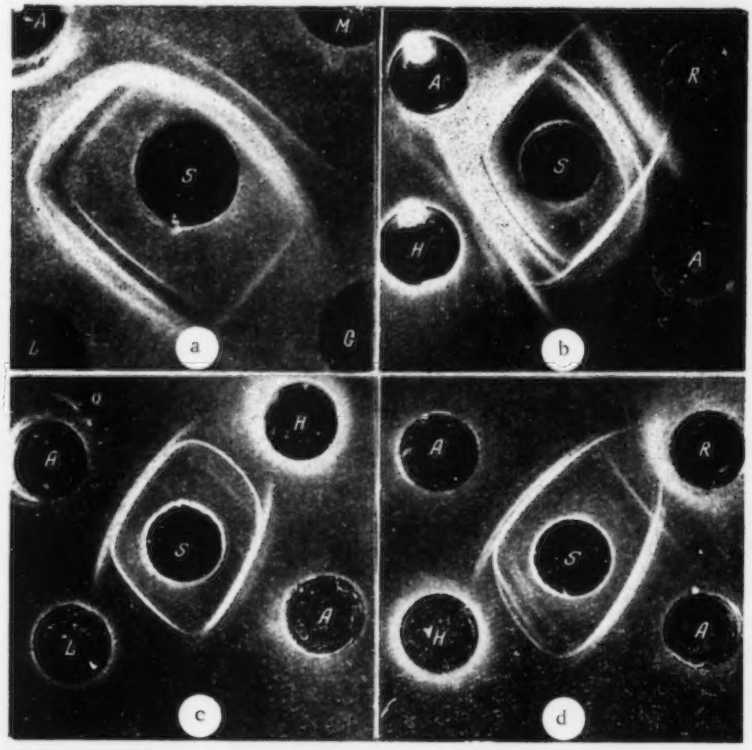

Fig. 1. Detection of specific and normal tissue and species antigens of Ehrlich's adenocarcinoma. a, b) Common antigenic behavior of tumor antigens, serum and spleen, liver and kidney of a normal mouse; c, d) detection of specific and normal tissue antigens of the tumor (adsorption by normal mouse serum in jelly). S) Antiserum to whole cells of Ehrlich's ascites adenocarcinoma; A) tumor; G) tumor globulin; R) kidney; M) mouse serum; H) liver; L) mouse spleen.

EXPERIMENTAL RESULTS

The antigens of the saline extract of the tumor were studied first. The antiserum to the tumor (Fig. 1, a, b) gave a series of common lines between the saline extract of the tumor and the antigens of normal organs: saline extracts of spleen, liver and kidney, and mouse serum. This suggests that the tumor contained a series of antigens common to the antigens of normal organs of mice.

After carrying out specific inhibition of precipitation with mouse serum, we found that most of the common lines disappeared. We called these disappearing antigens, serologically identical with serum proteins, conditionally species antigens. The antigens detected after specific inhibition of precipitation with mouse serum we called tissue antigens.

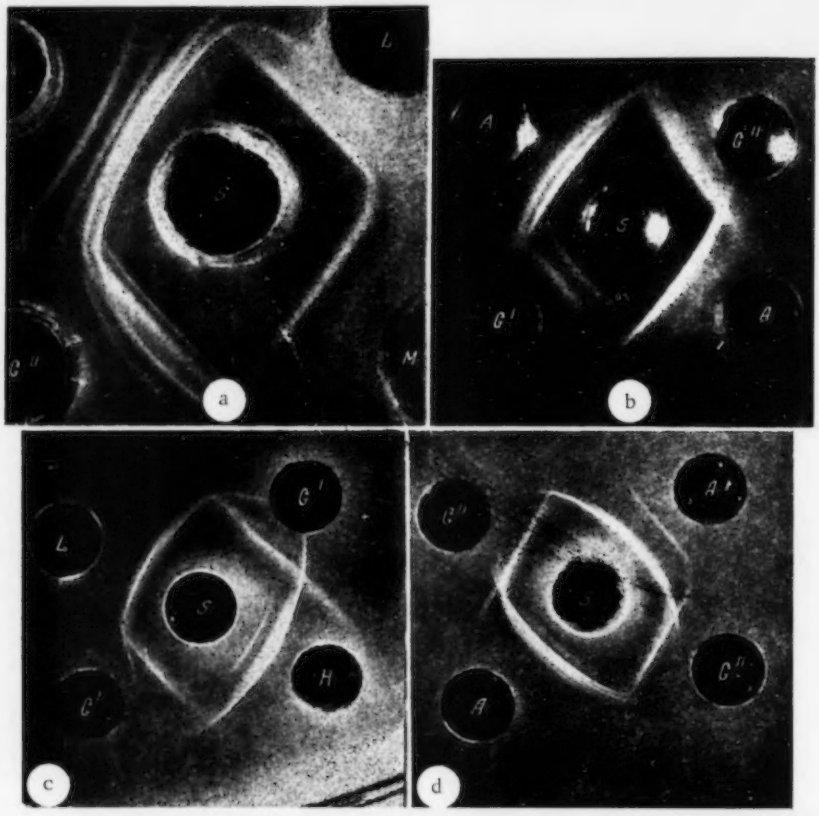

Fig. 2. Detection of tissue and species antigens of the globulin fractions of Ehrlich's adenocarcinoma. a, b) Common antigenic behavior between globulin fractions of the tumor and the saline extract of the tumor, the spleen and the serum of a normal mouse; c, d) detection of the tissue antigen of the globulin fractions of the tumor (adsorption by normal mouse serum in jelly). G', G") globulins I and II of the tumor. Remaining signs as in Fig. 1.

It is clear from Fig. 1, c, d that after saturation of the jelly with mouse serum, the saline extract gave two lines with antiserum to the tumor, suggesting the presence of two tissue antigens, differing from the serum, species proteins. One of these antigens was also present in the spleen and liver of the control mouse. The other antigen gave a clear additional line and was not present in normal organs.

Both these tumor antigens were absent from mouse kidney.

Vaccination of White Mice with Fractions of Ehrlich's Adenocarcinoma

Antigen	No. of animals	Total No. of tumors	No. of unsuccessful inoculations	Percentage of immune animals	Significance of differences between proportions of successful inoculations		Mean weight of inoculated tumors (in g)	Inhibition of growth of tumor (in %)
					χ^2 (comparison with controls)	χ^2 (comparison with vaccination in tail)		
Saline extract of tumor .	58	54	9	17	8.5	10	0.43	50
Globulin I of tumor. . . .	40	35	8	23	10	4.3	0.50	43
Globulin II of tumor . . .	43	41	9	22	12	5.3	0.55	45
Spleen	43	26	1	4	0.12	1.57	0.88	0
Globulin of liver	43	33	3	9	2.58	10	0.44	56
Vaccination in tail	72	46	22	48	28.8	–	0.27	69
Control	55	51	0	0	–	28.8	0.87	0

Note. The results of the three series of vaccination experiments are summarized in the table. The value of χ^2 was calculated from the "fourfold" formula [7]:

$$\chi^2 = \frac{(ad - bc - \frac{1}{2}k)^2 \cdot k}{e \cdot f \cdot g \cdot h}$$

for $p = 0.05$, when $n = 1$, and $\chi^2 = 3.81$.

Titration experiments in jelly, using dilutions of antigen increasing in multiples of two, before and after specific inhibition of precipitation by mouse serum showed that the content of species antigens in the saline extract was twice as great as that of tissue antigens.

We next studied the antigenic structure of the globulin fractions of the tumor (globulin I and II), obtained by salting out with 40% and 50% saturated ammonium sulfate respectively [2].

Antiserum to the tumor (Fig. 2, a, b) gave a series of lines common to the globulin fractions of the tumor and saline extracts of the tumor, spleen and liver, and mouse serum. This suggests the presence of a series of common antigens in the material tested. As the experiments with specific inhibition of precipitation by mouse serum showed, this common antigenic behavior was chiefly due to the presence of serum, species antigens.

After saturation of the jelly with mouse serum, a tissue antigen was found in the globulin fractions (Fig. 2, c, d). This was serologically identical with the tissue antigen of the saline extract of the tumor and the spleen. We have not yet been able to establish to which of the two tissue antigens of the tumor that we found the tissue antigen of the globulin corresponds, nor whether it is a specific tumor antigen or a normal tissue antigen [1, 5, 11].

We thus found by means of the precipitation reaction in jelly that 1) Ehrlich's adenocarcinoma and its globulin fractions possess a number of common antigens with normal organs of the mouse, principally serum, species antigens; 2) the tumor possesses at least two tissue antigens differing from species antigens; one of them is specific for the tumor alone, the other is identical with the tissue antigens of the liver and spleen of the control mouse; 3) the globulin fractions have one tissue antigen identical with the tissue tumor antigen and the tissue antigen of mouse spleen.

The results of the three series of experiments on vaccination of white mice are shown in the table.

It will be seen from the table that the highest resistance was given by vaccination with the globulin fractions of the tumor (globulin I and globulin II)—23% and 22% of immune mice respectively—and the saline extract of the tumor—17% of immune mice. The statistical significance of the differences in the proportions of successful inoculations by comparison with the control group was very high: the values of χ^2 were 10, 12, and 8.5, respectively.

Immunization with the globulin of normal mouse liver (specificity control) caused resistance in 9% of mice; $\chi^2 = 2.58$. Vaccination by means of implantation of the tumor in the region of the tail caused a considerable increase in the resistance of the mice to subsequent inoculation of the tumor (48% of immune mice, $\chi^2 = 28.8$).

In the control group the tumor was successfully inoculated in all the animals. The figures for the mean weight of the inoculated tumors showed basically the same relationship. Vaccination with a saline extract of the tumor inhibited development of the inoculated tumors by 50%, vaccination with globulin I—by 43%, and with globulin II of the tumor—by 45% by comparison with the controls.

Vaccination with liver globulin of the control mouse inhibited growth of the tumor by 56%. Immunization with spleen affected neither the growth nor the success of inoculation of the tumors. Vaccination in the tail inhibited growth of the developing tumors by 69%.

Analysis showed that the differences between the rate of successful inoculation and the mean weight of the tumors in the groups vaccinated with the globulin fractions and with the saline extract of the tumor were not statistically significant. The vaccinating activity of the antigens of the globulin fraction of the tumor was thus not inferior to the vaccinating activity of the original saline extract of the tumor.

The absence of vaccinating effect after inoculation of spleen was contrary to the findings of Khaletskaya [6] and other authors, possibly because we used lyophilized preparations. The high vaccinating effect of the globulin of normal liver may be due to the fact that an important part in the relative immunity observed is played by a nonspecific component, in the form of normal species and tissue antigens. The question of the role of the normal antigenic component in immunity to tumors demands further study.

The efficacy of vaccination by means of implantation of the tumor into the tail significantly exceeds the efficacy of vaccination by all the other tumor fractions (see table). This result may evidently be explained by the fact that the products of tumor metabolism entering the blood stream from this area of vaccination are more highly antigenic than the tumor fractions, which have been subjected to comparatively gross chemical treatment. These results support the view [3, 4] that a living vaccine is more effective than a chemical vaccine. We are convinced, however, that the use of milder methods of isolation of the tissue antigens, and of other schemes and doses of vaccination will greatly increase the efficacy of anti-tumor vaccination.

We thus were able to demonstrate the presence of at least two tissue tumor antigens in a saline extract of the tumor, differing from serum antigens, one of which was specific for the tumor. In the globulin fractions one tissue antigen was found. The globulin fractions were not inferior to the saline extract of the tumor in vaccinating activity. Vaccination with the living tumor was more effective than chemical vaccination with fractions of the tumor.

SUMMARY

The author studied the antigenic structure of Ehrlich's adenocarcinoma and its globulin fractions in reaction of precipitation in gel. Two tissue antigens were detected in the tumor, both differing from the species antigens; one of these tissue antigens proved to be specific. One tissue antigen was found in the globulin fractions. The latter showed pronounced immunizing activity in experiments with vaccination of mice. Inoculation into the tail with a living tumor was more effective than chemical vaccination with tumor fractions.

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THE STRUCTURE OF THE REGENERATING PAROTID SALIVARY GLAND IN RATS

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Of the large number of studies of regeneration of different viscera, comparatively few deal sufficiently with the structure of the regenerating organ. Also, most of these studies need to be reviewed in the light of new results concerning the regeneration of the viscera in mammals. According to these results, most of the viscera do not regenerate by growing out from a wounded surface, as had been supposed until now, but by a cellular proliferation of all parts of the organ. On this account, the mode of regeneration which M. A. Vorontsova has called regenerative hypertrophy [4], the organ may completely recover its weight while preserving its specific structure.

Despite the widespread occurrence of regenerative hypertrophy, it is not yet known in detail how it is brought about or what structural rearrangements occur. It remains to be found whether there is a new formation of structural units, or whether cellular proliferation brings about merely hypertrophy of existing units; we do not know whether there is merely a hyperplasia of the cells or whether at the same time there is a hypertrophy and a change in the relationships between the nucleus and cytoplasm, etc.

From the small amount of data available we know that regenerative hypertrophy occurs differently in different organs. After removal of two-thirds of the rat liver, the intensive proliferation of the liver cells which follows causes hypertrophy of the lobules, so that they are increased about two-fold. The hypertrophy is maintained for a long time [11]. In the regenerating rat kidney [10] a similar increase in the size of the structural units (nephrons) occurs. The course of events is somewhat different in the hypertrophy of the spleen. In mice, according to L. D. Liozner and G. V. Kharolva [8], not only do the Malpighian bodies hypertrophy, but they also increase in number. From one tenth portion of the spleen they were reported to increase in numbers by two and a half times.

Until recently it was thought that regeneration in the salivary glands occurs by the formation of atypical glandular tissue from the wounded surface, and that it starts from cells of the salivary ducts [5, 7, 9, 12, 13, 14]. Therefore, all descriptions of the structure of the regenerating gland concern only the way in which this atypical growth occurs. The differences between the different investigators concerned mainly their estimates of the functional properties of the newly formed terminal portions. Because we have shown that the parotid gland of guinea pigs [1] and rats [2] regenerates chiefly through a regenerative hypertrophy, and that the formation of atypical outgrowths is not of great importance, the question arises as to how the structure of the gland changes during regeneration.

In the work reported here we set out to find whether the size of the terminal portions and the number of cells which compose them increase, and we hoped thereby to be able to determine whether the regenerating gland becomes restored through a process of hypertrophy or through the formation of new terminal portions.

METHOD

The work was done on seven regenerating parotid salivary glands of white rats. The glands were extirpated 1½-2 months after removing the whole of one and approximately half of the other parotid gland. The weight of these glands was approximately the same as that of glands in control animals, which indicated that regeneration had occurred. The control glands were taken from intact rats of the same age and weight (close control).

The glands were fixed in formalin, and treated by normal histological methods. The sections were stained by Gomori's method. In each gland a count was made of the number of terminal portions per 100 fields of view; the magnification was 90 × 7 and the field was defined by a square window in the eyepiece measuring 7 × 7 mm. A count was also made of the number of nuclei present in each terminal portion.

The results of the count are shown in the Table.

It can be seen from the Table that there is no great difference between the number of terminal portions in the control and experimental gland, the numbers being 490 and 450 respectively. There is no statistically significant difference which shows that with our method of performing the experiment the terminal portions do not hypertrophy, and there is no increase in the number of cells in the different terminal portions. A count of the number of nuclei in the terminal portions showed that it varies greatly, but that this variation occurs both in the normal and in the regenerated gland. The difference in the average number of nuclei per terminal portion is not great: it varies from 6.9 to 11.9 in the experimental gland, and from 6 to 10.7 in the control. It can be shown statistically that the difference between the mean numbers of nuclei in the experimental (9.1) and control (8.1) glands lies within the range of possible chance variation ($P = 0.12$).

Number of Terminal Portions and Nuclei in a Regenerated and in a Normal Rat Parotid Salivary Gland

Regenerated gland		Intact gland	
number of terminal portions per 100 fields of view	mean number of nuclei per terminal portion	number of terminal portions per 100 fields of view	mean number of nuclei per terminal portion
288	10,9	436	6,9
461	11,6	480	8,8
682	7,6	505	8,2
644	8,0	589	9,3
717	6,9	458	7,3
381	8,8	252	6,0
319	9,8	358	10,7
—	—	498	7,9
mean 490	9,1	450	8,1

From these experiments it may be concluded that after complete removal of one parotid and half the other because the size of the terminal portions remains unchanged and yet the mass of the gland increases 2-3 times that the regenerative hypertrophy occurs not through the growth of existing terminal portions but through formation of new ones. Naturally, it is possible that with some other arrangement of the experiment (as for example by removing a still greater amount of glandular tissue) there might be some growth in the terminal portions in addition to an increase in their numbers, though the possibility of this occurring is not great. In any case, it should be noted that when a similar proportion of liver or kidney is removed, there is not only hyperplasia, but also a considerable cellular hypertrophy. The results we have obtained, therefore, indicate that regenerative hypertrophy proceeds differently in different organs.

In certain organs, hypertrophy of cells and structural units of the organ plays an essential part in regeneration (as in the liver and kidney); in others, such as the salivary gland, there is no hypertrophy, but new cells and new terminal portions are formed. It appears that regeneration takes a similar course in the spleen [8] and in the small intestine [3].

SUMMARY

The number of terminal portions and of their nuclei in 100 fields of view were counted in normal and in regenerating parotid glands. There was no difference either in the number of terminal portions or in the nuclei in them. This result suggests that regeneration of the salivary glands occurs by the formation of new end portions within the boundaries of the remaining portion of the gland, and not as a result of an increase in their size.

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REGENERATION OF THE ISLET TISSUE IN VARIOUS CONDITIONS OF DIET AND IN HYPO- OR HYPERTHYROIDISM

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Much research has been devoted to the study of the regeneration of the exo- and endocrine divisions of the pancreas in different experimental conditions [1-7]. Nevertheless, the problem of the intraglandular relationships between the acinose and islet cells in the course of the reparative regeneration of the pancreas has remained unsolved until recently. No unanimity exists regarding the ways and means of the formation of new islet tissue at the place of injury.

In the present report, which is not concerned with the methods of restoration of the exocrine epithelium, we present information concerning the sources of formation of the islet tissue of the regenerating pancreas under different conditions of diet and with marked variation in the concentration of thyroid hormone.

EXPERIMENTAL METHOD

White rats were used as experimental animals. For 12 days before the experiment the rats were kept on an ordinary (mixed) diet or on a carbohydrate or protein diet. The greater part of the tail of the pancreas was removed from all the animals. In control experiments we studied the regeneration of the islets in animals receiving no form of medication. The experimental rats began to receive thyroidin or 6-methylthiouracil five days before partial pancreatectomy, and administration continued throughout the period of observation. The daily dose of thyroidin for each animal was 3 mg, and that of 6-methylthiouracil 5 mg. Material for investigation was taken 5 and 15 days after operation. The tissue was fixed in Zenker's or Bouin's fluid and embedded in paraffin wax. Sections were stained with hematoxylin and eosin, chrome-hematoxylin-fuchsin by Gomori's method, aldehyde-fuchsin and methyl green with pyronine. The number and size of the islets in the control and experimental groups were compared by measuring the diameter of the islets in the histological preparations and calculating the total area of islet tissue per unit area of the parenchyma of the gland as a whole. For this purpose in each experimental series of animals the outlines of the whole area of the histological section and also the outlines of the individual islets in 50 sections were traced on paper by means of the RA-4 drawing apparatus. In making the calculation the true magnification of the microscope was taken into consideration, and the areas were expressed in absolute figures.

EXPERIMENTAL RESULTS

Measurement of the diameter of the islets and calculation of their area showed that the total mass of islet tissue in the areas of the pancreas left behind after partial pancreatectomy varied with the experimental conditions (see table)*

* The differences in the area of the islet tissue in the different series of experimental animals shown in the table were statistically significant. The differences between the series of animals kept on different diets were also significant.

Changes in the Islet Tissue in the Regenerating Pancreas and in the Residual Part of the Organ (15 Days after Operation) in Different Conditions of Diet and with Variations in the Concentration of Thyroid Hormone

Group (diet)	Series	Mean diameter of islet (in μ)	Relative area of islet tissue per 1 mm² of the glandular parenchyma of the organ
Mixed	Partial pancreatectomy (control)	83.8	0.254
	Partial pancreatectomy + thyroidin	93.6	0.275
	Partial pancreatectomy + methylthiouracil .	84.5	0.215
Carbohydrate	Partial pancreatectomy (without medication)	92.6	0.344
	Partial pancreatectomy + thyroidin	125.4	0.477
	Partial pancreatectomy + methylthiouracil .	95.6	0.264
Protein	Partial pancreatectomy (without medication)	68.6	0.198
	Partial pancreatectomy + thyroidin	82.3	0.210
	Partial pancreatectomy + methylthiouracil .	80.8	0.201

It will be seen from the figures in the table that in the animals of the "carbohydrate" group the dimensions and the total area of the islets increased considerably by comparison with the other two groups. This was most marked in the rats receiving thyroidin, in which the total area of the islets was almost twice that of the control animals 15 days after partial pancreatectomy. In animals on a protein diet very weak development of the islet tissue was observed.

A study of the sections of the pancreas from animals of the different experimental groups and series confirmed the findings described above. The formation of islet tissue in the residual and the newly regenerating areas of the pancreas was observed in both control and experimental animals. The intensity of restoration of the intrasecretory portions of the gland at the site of the injury, and of the compensatory phenomena in the areas of the organ remaining after operation, followed a different course, however, in the various experimental conditions.

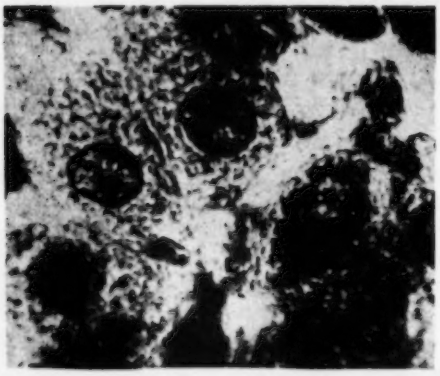

Fig. 1. Acinus containing three islet cells. Pancreas of a rat 5 days after operation. Carbohydrate diet. Magnification 1200 ×.

In the control animals kept on an ordinary mixed diet and receiving neither thyroidin nor methylthiouracil, the regeneration of islet tissue at the site of injury was effected by means of epithelial tubes and bands, which were formed as a result of the reorganization and proliferation of the cells of the acini and the cells of the small efferent ducts. These islets consisted of a small number of cells in direct association with the cells of the tubules or efferent ducts. In the residual portion of the gland the regeneration of islet tissue took place mainly by the conversion of acinose into islet cells. Mitoses were found very rarely in the islet cells. Among the acinose tissue individual cells were found with the typical granules and nuclei of islet cells. The structure of the acinose cells situated at the periphery of the islets was frequently modified; intermediate structures between the typical exocrine and islet cells were found. The cytoplasm of these cells in some cases was characteristic of the islets, and the nuclei still resembled those of the acinus cells. Cells were found with nuclei typical of islet cells, but with cytoplasm containing individual granules of zymogen. It must be pointed out that the appearance of new islet cells at the site of injury and in areas of the gland away from the defect always took place where there was an abundant blood supply.

A carbohydrate diet stimulated the formation of islet tissue; it stimulated in particular the process of formation of new islet cells in the part of the gland remaining after the operation. In animals on a nonprotein diet, in areas of the gland remote from the site of injury there were many small islets containing 2-5 cells. Islet cells, developing from acinus cells, were in close contact with the cells of the terminal portions of the gland or formed part of the acini themselves (Fig. 1). At the periphery of the islets (with a diameter from 51 to 87 μ) transitional structures were observed (Fig. 2) far more often than in the animals kept on an ordinary mixed diet. As a result of this formation of islet cells from acinus tissue in the rats of the "carbohydrate" group the islets enlarged and increased in number.

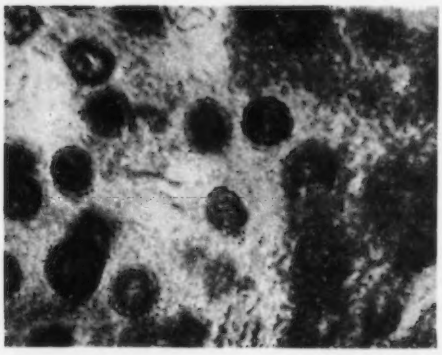

Fig. 2. Formation of new islet cells from adjacent acinus tissue. Pancreas of a rat 5 days after operation. Carbohydrate diet. Magnification 760 ×.

Keeping the white rats on a protein diet did not cause such an intensive formation of islet cells in the portion of the gland left after partial pancreatectomy. The conversion of acinus tissue into islet, and the development of new islet cells from the epithelial tubes and bands were more rarely found in animals on a protein diet than in rats kept on a mixed or carbohydrate diet. The feeble development of the islet tissue in the pancreas in rats receiving a diet with a high protein content and subjected to partial pancreatectomy was evidently caused by the lowered insulin requirement of the animal by comparison with that of animals of the "carbohydrate" group.

A change in the concentration of thyroid hormone in the experimental animals kept on different diets was, in turn, reflected in the regeneration of the islet tissue of the pancreas. The process of formation of new islets in the regenerating portion of the gland was effected after administration of thyroidin, like in the control animals, by two methods: from the epithelial tubes and bands formed as a result of the reorganization and proliferation of the cells of the acini and of the intermediate divisions and of the cells of the efferent ducts, and in the residual part of the organ – mainly by means of the conversion of acinus tissue into islet tissue. In the animals subjected to the action of thyroidin, however, the total area of the endocrine part of the gland was slightly larger than in the controls. This increase in the total mass of islet tissue in the rats receiving thyroidin was associated with the intensive proliferation of epithelial structures in the region of the injury, as a result of which the formation of many small islets was observed. The conversion of acinus tissue into islet tissue was more obvious in the areas of the gland remote from the place of the defect. For this reason islets of large size were often encountered in the residual portion of the head of the pancreas.

A considerable increase in islet tissue took place in animals kept on a carbohydrate diet and receiving a small dose of thyroidin. In these conditions, at the periphery of the islets we observed in large numbers not only individual acinose cells undergoing conversion into islet cells, but also complete large acini of a modified structure (Fig. 3). Such acini contained cells at different stages of conversion of exocrine tissue into islet tissue. Under these circumstances the cells turning into islet cells had the characteristic cytoplasm of islet cells; the cells situated near the acini, however, still contained a homogeneous, basophilic zone of cytoplasm. The incretory activity of the islet cells in animals on a carbohydrate diet and receiving thyroidin was greatly intensified. This was shown by an increase in the number of α-cells in the large islets and the large number of small islets containing only β-cells. The marked increase in function of the islet tissue could be explained by the increased insulin requirement of the animal, caused by the partial pancreatectomy, the stimulation of the endocrine tissue, the carbohydrate diet and the administration of thyroidin. It may be assumed that the excessive stimulation of the islet tissue (more prolonged maintenance of the animals on a protein-free diet than in our experiments and the use of large doses of thyroidin) would lead not to an increase in the regenerative power of the islets, but to their functional exhaustion.

A protein diet, coupled with administration of thyroidin, did not lead to the intensive formation of islets. In such conditions the total area of islet tissue 15 days after partial pancreatectomy was always much smaller than when the animals were kept on a carbohydrate diet.

The regeneration of the islet tissue in the animals of the different experimental groups when an inadequate concentration of thyroid hormone was present was effected mainly in the part of the gland remaining after the operation, for the proliferative processes at the site of injury were strongly depressed. In response to the administration of 6-methylthiouracil, islets were formed most frequently of all from the epithelium of the small efferent ducts, and only here and there were islet cells observed to be formed from acinus tissue.

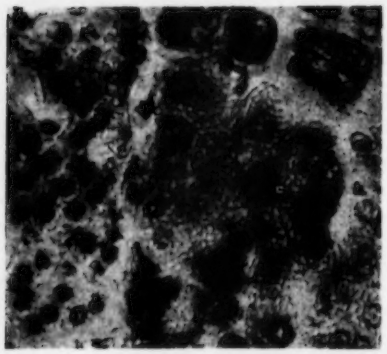

Fig. 3. Acinus of modified structure, situated near an islet. Pancreas of a white rat on a carbohydrate diet and receiving thyroidin, 5 days after operation. Magnification 360 ×.

When taken as a whole, our findings indicate that the source from which islet tissue is formed in the pancreas of rats may be both the reorganizing and proliferating epithelium of the acini and cells of the small efferent ducts, and also the acinose tissue itself of that organ. The process of the formation of new islet tissue varies with the character of the diet and the level of the basal metabolism, as determined by the concentration of thyroid hormone in the body.

SUMMARY

A study was made of the sources of insular tissue formation in the pancreas of albino rats during its restoration in conditions of different diet and changes of the thyroid hormone concentration. The reconstructing and proliferating acinus epithelium, the cells of small efferent ducts and acinus tissue of the remaining parts of the organ proved to be the source of the insular tissue formation. The process of the new insular tissue formation was the most intense in conditions of carbohydrate nutrition and thyroidization.

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THE MECHANISM OF REGULATION OF THE GROWTH OF ORGANS IN EMBRYOGENESIS

COMMUNICATION II. THE ACTION OF SPLENIC TISSUE TRANSPLANTS FROM CHICK EMBRYOS, ENLARGED UNDER THE INFLUENCE OF ADULT SPLEEN ON GROWTH OF THE ORGANS OF NORMAL CHICK EMBRYOS

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In our previous communication [2], and in work by other authors [4-9], it has been shown that organs transplanted on to the chorioallantois of chick embryos stimulate growth of the corresponding organs in these embryos.

Those writers who have attempted to explain the mechanism of this phenomenon have adopted two principal points of view. According to one of these [1, 3, 5], substances from the transplant penetrate into the corresponding organs of the embryos and stimulate the biosynthesis of proteins. The most widely held view at the present time, however, is [7] that whole cells from the transplant of the spleen growing on the chorioallantois pass into the spleen of the recipient embryo with the blood stream. As a result of the action of the antigens of the embryonic tissues, these cells produce antibodies, which cause pathological hypertrophy of the corresponding organs of the embryos.

Action of Transplants of Splenic Tissue of Chick Embryos, Enlarged under the Influence of Adult Spleen, on Growth of the Organs of Chick Embryos of 10 Days of Incubation

Time during which spleen of embryos was under influence of spleen of adult fowl	No. of cases	Mean weight of organs (in mg)					
		spleen		heart		liver	
		in absolute figures	as percentage of controls	in absolute figures	as percentage of controls	in absolute figures	as percentage of controls
2 days	21	21,43	**252,1**	110,3	121,6	322,24	**144,2**
(control)	9	8,5	100	90,3	100	223,4	100
4 days	22	71,0	**606,8**	174,7	**123,6**	688,4	**186,7**
(control)	18	11,7	100	141,3	100	368	100
7 days	31	60,8	**520**	162,4	115	647,6	**175,7**
(control)	18	11,7	100	141,3	100	368	100

Note. The figures indicating statistically significant changes in the weight of the organs are framed.

In our first communication [2] we showed that the degree of stimulation of growth of the embryonic organs under the influence of the transplants, and the specificity of their action are dependent on both the age of the recipients and the age of the donors. It was also shown that pieces of the spleen of chick embryos, incapable of producing antibodies, stimulate growth of the organs of recipient embryos.

These findings evidently conflict with the views of Simonsen [7], for immunized spleen cells from adult fowls (by Simonsen's method) would be expected to act identically on embryos of different periods of development. The cells of embryonic spleens, which are incapable of producing antibodies, would be expected to have no action whatsoever on growth of the organs of recipient embryos.

Simonsen's views were thus not confirmed, and the question of the mechanism of the growth-stimulating action of the transplants is still in effect unanswered.

In an attempt to solve this problem, we undertook special investigations. In the present communication we describe the results of some of these investigations, in which we studied the action of pieces of embryonic spleen, which had increased in size under the influence of previously transplanted pieces of the spleen of adult fowls, on the growth of the spleen in normal embryos.

EXPERIMENTAL METHOD

As recipients we used chick embryos incubated for 10 days. The donors were embryos of 12, 14, and 17 days of incubation. The experiments were carried out as follows. Pieces of the spleen of adult fowls were transplanted on to the chorioallantois of chick embryos of 10 days of incubation. The spleens of the embryos, which had grown in size under the influence of the transplants, were extracted 2, 4, and 7 days after transplantation, and pieces of them were again transplanted on to the chorioallantois of 10-day embryos. The experimental method is described in detail in the first communication [2].

EXPERIMENTAL RESULTS

The results of the experiments are presented in the table.

It may be seen from the table that after transplantation of pieces of the spleen of chick embryos of 12 days of incubation, which had previously been exposed for two days to the influence of transplants of the spleen of adult fowls, a considerable increase in the growth of the spleen took place (by 152.1%), together with a small increase in growth of the liver (by 44.2%) of the recipient embryos. No significant change in the weight of the heart took place.

After transplantation of the spleen of chick embryos of 14 days of incubation, previously exposed to the influence of transplants of the spleen of adult fowls for four days, a considerable increase in the growth of the spleen (by 506.8%) and a small increase in the growth of the heart (23.6%) and liver (86.7%) were observed in the recipient embryos.

After transplantation of the spleen of chick embryos of 17 days of incubation, previously exposed to the influence of transplants of the spleen of adult fowls for seven days, considerable stimulation of growth of the spleen (by 420%) was also observed. Growth of the liver was also stimulated, although not to the same extent (75%). The growth of the heart was essentially unaltered.

Thus, the embryonic spleen, when enlarged under the influence of transplants of adult spleen, stimulates very considerably the rate of growth of the spleen of recipient embryos, and to a much lesser degree stimulates the growth of other organs.

As we showed previously [2] the spleen of adult fowls and the spleen of chick embryos stimulate growth of the spleen of recipients. The stimulation in these cases, however, was expressed to a far less degree than that of transplants of the spleens of embryos previously exposed to the action of transplanted pieces of adult spleen.

It is obvious that the number of cells of the adult spleen in the enlarged spleens of the embryos cannot be greater than in the transplants of adult spleen. Even if the number of cells introduced from the transplant into the modified tissues of the spleens (as suggested by Simonsen [7]) was equal to the number of "adult" cells in the "adult" transplant itself (which, of course, is unlikely), then in this case the rate of growth of the spleen could not be as high as took place in our experiment. The results which we obtained cannot, therefore, be explained

on the basis of Simonsen's views. On the contrary, it may be postulated from our findings that under the influence of substances passing from the organ transplants into the same organs of the recipient embryos, a modification of the processes of biosynthesis takes place in the latter, which leads to stimulation of the processes of growth.

SUMMARY

Pieces of spleen of adult hens were transplanted onto the chorionallantoic membrane of chick embryos (10 days of incubation). Enlarged spleens of these embryos were again transplanted onto the chorionallantoic membranes of embryos-recipients (10 days of incubation). Transplantation of pieces of such spleens led to very strong growth stimulation of the spleens in embryos-recipients.

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HISTOCHEMICAL INVESTIGATION OF THE NUCLEI OF SUBCUTANEOUS CONNECTIVE TISSUE CELLS OF WHITE RATS EXPOSED TO THE ACTION OF ULTRASOUND

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Translated from Byulleten' Éksperimental' noi Biologii i Meditsiny, Vol. 51, No. 5, pp. 101-103, May, 1961
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In view of the increasing application of ultrasound in the treatment of a number of diseases, we considered that it would be of interest to investigate its effect on connective tissue, because this tissue evidently reacts to such treatment.

EXPERIMENTAL METHOD

As ultrasound generator we used the Zonostat-515 apparatus with a piezo-quartz pick-up (not water-cooled), the active surface of the vibrator being 7 cm^2, and giving a continuous supply of ultrasound waves with a frequency of 800 cps. In view of the small size of the animal, we used a stable method of application (through a tube of water). As a rule, two applications were given (2 minutes each, on alternate days) to the inguinal region. Immediately after the second exposure of the animals to ultrasound, and subsequently after 3, 10, and 30 days, fine films of subcutaneous connective tissue were taken from the superficial layer of the inguinal and axillary (not exposed to ultrasound) regions. The connective tissue films (7 films from each area) were prepared by Maximow's method, fixed in Helly's mercuric chloride solution, and stained with iron-hematoxylin by Yasvoin's method.

EXPERIMENTAL RESULTS

Examination of preparations (50 fields of vision in each) from films taken immediately after the second exposure to ultrasound of relatively high intensity (1.17 W/cm^2) showed profound changes in the connective tissue structures: homogenization of the ground substance, a marked decrease in the number of cells, and the presence of only 2-3 "bare", irregularly shaped nuclei in a field of vision or their complete absence. In the case when ultrasound of comparatively low intensity (0.2 W/cm^2) was used, the changes were much slighter in degree: the number of cells was 10.1 (compared with 18 in the controls), and in half of them the nuclei were irregular in shape; the protoplasm was preserved on the average in only two cells. We also observed swollen collagen fibers, a few elastic fibers, and dilated capillaries.

The pattern of the changes in the axillary region was similar but less marked.

After the initial depression of the tissue, during the first few days of the after-effect processes of recovery of the connective-tissue structures were observed (an increase in the number of cells and normalization of the fibrous structures). When ultrasound with an intensity of 1.17 W/cm^2 was used, however, the process of recovery

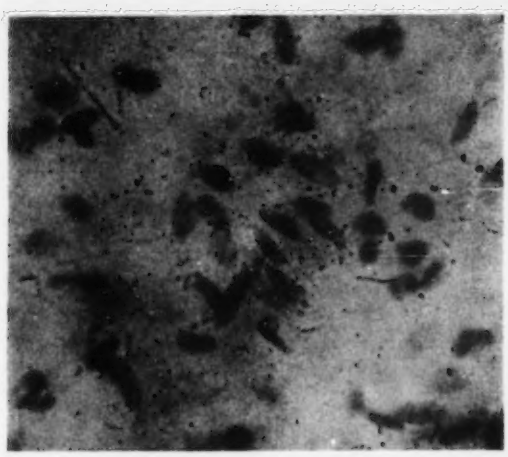

Fig. 1. Subcutaneous connective tissue of a white rat, not exposed to ultrasound. Stained with fuchsin. Magnification: ocular 15 ×, objective 40 ×.

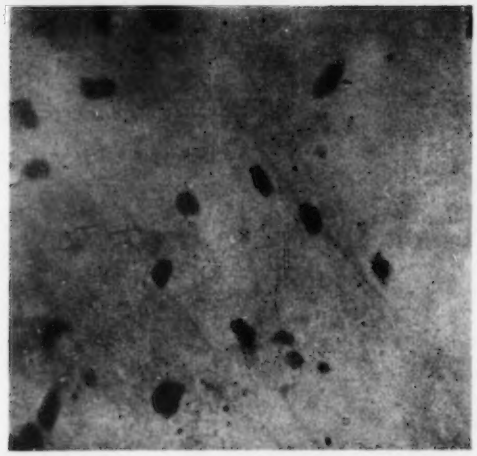

Fig. 2. Subcutaneous connective tissue of a white rat one month after the second exposure to ultrasound (intensity of irradiation 1.17 W/cm^2). Stained with fuchsin. Magnification: ocular 15 ×, objective 40 ×.

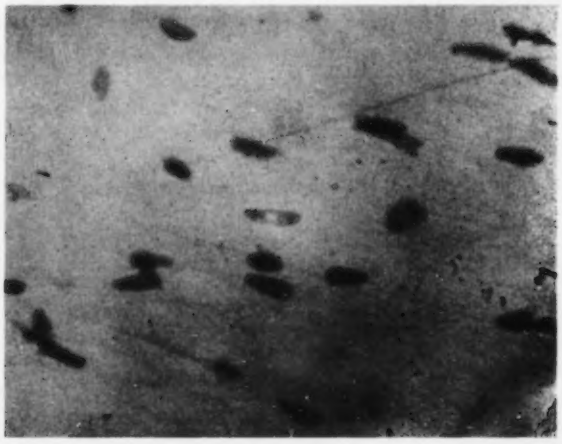

Fig. 3. Subcutaneous connective tissue of a white rat one month after the second exposure to ultrasound (intensity of irradiation 0.2 W/cm^2). Stained with fuchsin. Magnification: ocular 15 ×, objective 40 ×.

of the connective-tissue structures was not complete on either the 10th or the 30th day after exposure. After the action of ultrasound with an intensity of 0.2 W/cm^2 the phase of initial moderate depression of the tissue quickly gave way to a phase of restoration and renovation of the tissue. In preparations from films of tissue taken 10 days after exposure, evidence of stimulation of the vital activity of the tissue was seen. This was especially noticeable one month after exposure: an increase (by comparison with the controls) in the number of cells with more intensively stained protoplasm than the controls; an increase in the number of young cells and of mast cells; the predominance of elastic fibers among the fibrous structures, which is characteristic of younger tissue.

There are reports in the literature [1, 2, 3, 4] of the stimulating effect of small doses of ultrasound on various organs and tissues of the intact animal.

The histological evidence which we obtained of the stimulation of connective tissue by small doses of ultrasound suggests an increase of its functions and an intensification of its vital activity, which may be associated with an alteration in and a stimulation of the biochemical processes of metabolism within the tissues and cells. We accordingly considered it important to study the effect of ultrasound on certain processes of the metabolism of nucleic acids, and in particular of desoxyribonucleic acid (DNA).

We studied the DNA content of the subcutaneous connective tissue of white rats. The experimental conditions were the same as those described above. Experiments were carried out on 21 rats (adult females weighing 200-250 g), seven of which acted as controls. DNA was determined by Feulgen's method.

Relatively slightly stained cell nuclei are seen in Fig. 1 (control)*. When the changes in the connective tissue were studied one month after exposure to ultrasound in a dose of 1.17 W/cm^2, an increase in the intensity of staining of the cell nuclei was observed by comparison with the controls; this demonstrates intensification of the nucleic acid metabolism. It must be pointed out that when this dose was used only part of the nuclei was stained more brightly than in the control (Fig. 2). The intensity of staining of the nuclei was more marked one month after exposure to ultrasound of relatively low power (0.2 W/cm^2). In this case most nuclei were more intensively stained than in the control (Fig. 3).

The results obtained from our investigation of the influence of small doses of ultrasound on the DNA content of the connective-tissue cells thus also demonstrate that this effect is one of stimulation.

SUMMARY

The work involves histological and histochemical examinations.

Inguinal region of the animals was subjected to ultrasound action of relatively high (1.17 W/cm^2) and low (0.2 W/cm^2) intensity. Connective tissue membranes were prepared according to Maximov, and stained according to Yasvoin with iron hematoxylin. Since ultrasound may affect the metabolism of nucleic acid, the study of the dynamic changes in the amount of desoxyribonucleic acid, conducted for a period of one month after ultrasound treatment was made. The Felgen staining method was used to detect desoxyribonucleic acid.

Ultrasound of relatively low intensity caused connective tissue stimulation with changes in the nucleic acids metabolism (sum total rise of desoxyribonucleic acid content). Ultrasound dispensed in dose of 1.17 W/cm^2 caused prolonged depression of loose connective tissue function, and, apparently, some disturbances on the nucleic acid metabolism.

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* With the staining method used in the preparations illustrated, only the nuclei are stained.

THE INFLUENCE OF A SUPER-HIGH FREQUENCY PULSED ELECTROMAGNETIC FIELD ON CONCEPTION AND THE COURSE OF PREGNANCY IN WHITE MICE

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Scientific Directors: Corresponding Member AMN SSSR Prof. A. V. Triumfov and Prof. V. G. Butomo
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The rapid development of radioelectronics in the Soviet Union and the large part played by women in all branches of the national economy, including the manufacture of radio apparatus, make it essential to study the influence of the super-high frequency (SHF) field on the female organism, especially during pregnancy, and also on the course of pregnancy and on the fetus.

In an attempt to solve this problem, we carried out experiments on female mice exposed to the action of a pulsed SHF field. We studied the time of conception, the changes in the course of pregnancy, and the possible changes in or injury to the fetus in relation to the action of a low-intensity SHF field on the pregnant animal, and also the course of parturition in the mice and the development of their offspring.

EXPERIMENTAL METHOD

In the first series of experiments mice were exposed to the SHF field of power flux density 0.344 mW/cm^2 twenty times, each exposure being for 30 minutes (20 experimental and 10 control animals), and in the second series fifty times with the same power flux density and duration of exposure (20 experimental and 10 control animals). In these two series the animals were irradiated before copulation, and in the third series (35 experimental and 19 control mice) irradiation began immediately after copulation and continued during 12 days of pregnancy. The female mice were exposed daily for 10 minutes to a field of power flux density of 50 mW/cm^2.

The experimental animals were weighed and their condition was observed every day before, during, and after pregnancy. In some of the female mice the sexual cycle was investigated before copulation and three weeks thereafter for a prolonged period.

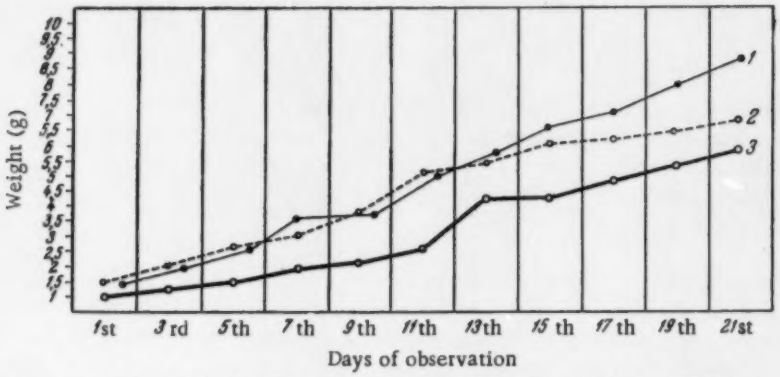
EXPERIMENTAL RESULTS

Prolonged exposure of female mice to the SHF field before copulation had no effect on the onset of pregnancy. In the experimental group pregnancy took place in 23 of the 40 mice (117 fetuses), and in the control group in 13 of the 20 mice (56 fetuses). The number of young mice in the litters of the experimental and control groups was the same.

Exposure to the SHF field was not followed by the termination of pregnancy or the onset of abortion. Only three experimental females showed great distress one or two days before the beginning of parturition. The weight curve of the experimental animals during the whole period of pregnancy showed a gradual increase, to reach a maximum average figure of 33.5-37.5 g when irradiation was given before pregnancy (38 g in the controls), and 33-34.5 g when irradiation was given during pregnancy (12 exposures, with a power flux density of 50 mW/cm^2).

Premature parturition was not observed in any of the experimental mice, but on the contrary there was a tendency for postmaturity to take place: parturition began on the 19th-21st day after the last day of copulation, by comparison with the 18th day in the control group. Among the postmature newborn mice there were four stillborn.

Postmaturity of the fetus may be due to a depression of the function of the afferent system of the uterus as a result of changes in the nervous system of the mouse brought about by exposure to the SHF field. The weakening of the influence and the change in the character of the stimuli coming from the fetuses and affecting the female mice [2, 4, 5] as a result of the action of the SHF field may also explain the later arrival of the fetuses than in the case of the control animals. There are reports in the literature of changes in the central nervous system of animals under the influence of the SHF field [1, 10]. During observation of the animals in the postnatal period no difference from the controls both as regards their general condition and as regards the condition of their organs could be observed.



Change in the weight of white mice under the influence of the super-high frequency field. 1) Control; 2) experiment, exposure before pregnancy; 3) experiment, exposure during pregnancy.

Exposure to the SHF field had a definite influence on the development of the young mice. On the 5th-6th day after birth the skin of the young of the experimental females began to acquire a silvery hue. They were covered with hair on the 9th-10th day (the controls on the 8th-9th day), and with a white fur on the 11th-13th day (the controls on the 9th-12th day). Usually on the 11th-13th day the eyes of the young mice had the appearance of perforated slits instead of the film as previously observed; the eyes were opened on the 14th-15th day (in the controls on the 13th-14th day). The development of the young mice born of the experimental females exposed to the action of the SHF field before pregnancy took place rather faster than in the young of the experimental females exposed to the SHF field during pregnancy.

The subsequent development of the young experimental mice followed the same course outwardly as that of the young of the control female mice. On the 19th day they became mobile and more fluffy, and they tried by themselves to drink and to gnaw grain, which they managed to do perfectly well on the 21st-25th day. The average weight of the fetuses at birth was 1.5 g (according to Kovalevskii [7], the average weight of newborn mice is 1.4-1.5 g, according to Robertson [9]—1.7 g).

At the end of the first week the average weight of the young of the females exposed to the SHF field during pregnancy reached 2.5 g, that of the young of the females exposed to the SHF field before pregnancy was 3.5 g, and that of the young of the control females 3.8 g. At the end of the second week equalization of the weight of the young of the females exposed to the SHF field before pregnancy (5.7 g) and the young of the control animals (6.2 g) took place. The weight of the young of the females exposed to the SHF field during pregnancy lagged behind considerably (4.7 g). At the end of the third week the average weight of the young of all the experimental females was approximately the same (6.7-7.1 g).

The newborn offspring of the control females developed well; their average weight at birth was between 1.5 and 2.0 g, at the end of the second week it reached 6.2 g, and at the end of the third week it was 9.2 g (see figure).

By comparison with the control series, some lagging behind may thus be observed in the weight of the young of the experimental females, especially those exposed during pregnancy.

In the experiments in which the females were exposed for long periods before pregnancy (the first and second), in the first two weeks three newborn mice died, and in the 3rd and 4th weeks 87 of the 117 young mice died. Abnormalities of the gastrointestinal tract, reddening of the eyes, loss of the hair, and general lethargy were observed in these animals. In the control group 11 of the 56 young mice died.

In the third series of experiments two young mice died on the eighth day after birth, two died on the 16th day, and three on the 17th day.

It must be supposed that the action of the SHF field at the beginning of pregnancy on the fetuses is much greater (they have a small birth weight, they lag behind considerably in weight and development, and a high proportion of them die in the third week after birth)*.

The reaction to chorionic gonadotropin was tested in three young mice born of females exposed to the SHF field before pregnancy (from the first two series). It gave a negative result. The analogous reaction in four young mice (from two litters) born of females exposed to the SHF field during pregnancy (third series) gave a negative result in one mouse and a positive in three (from the same litter). In all the young mice born of the control animals, the reaction to chorionic gonadotropin was positive.

Most of the experimental and control animals (whether dying or sacrificed) were examined post mortem. Particular attention was paid to the state of the sex organs (the ovaries and the uterine cornua); these were also examined histologically.

Visible pathologo-anatomical changes in the ovaries of the experimental mice were observed only in isolated cases, in the form of slight cystic degeneration and a decrease in their size and weight. The average weight of the ovaries of the control animals was 19.5 mg, and that of the experimental animals 11.2 mg. The uterine cornua of the experimental mice were less than half the size of those of the control animals. This decrease in the weight of these organs after exposure to the SHF field is evidently the result of atrophic changes.

The greatest weight of the uterine cornua of the experimental and control animals was observed in the stage of oestrus. A few of the sacrificed and dying mice had hemorrhages in the uterine cornua.

In the parturient females, congestion and hemorrhages were also observed in other organs, e. g. the lungs and liver. The uterine cornua of the control females were unchanged. Histological examination of the ovaries of individual experimental female mice revealed a comparatively small number of follicles and corpora lutea, and also some diminution in their volume; certain follicles had undergone cystic degeneration. In the uterine cornua of the experimental females no particular changes were found. In some of them, especially in the stage of oestrus, we observed dilated glands and a more compact interglandular connective tissue, rich in cells. In some experimental female mice no changes whatsoever were found. More significant changes by comparison with the controls were found in the female mice in the stages of pro-oestrus and oestrus than in the stages of metoestrus and dioestrus.

According to reports in the literature [3], the developing and developed Graafian follicles are more sensitive to X-rays than the primordial ovarian follicles. It has also been reported [6] that the ovaries are more sensitive to the action of ionizing radiation during a period of functional activity than during a period of relative functional rest.

The investigations of Pitenin [8] of the testes of rabbits exposed to the action of a weak SHF field (20 exposures, each of 60 minutes), revealed several morphological changes in the form of a slight decrease in the size of the sex cells, some shrinking of these cells, absence of more mature forms and, sometimes, the presence of degenerated cell groups.

Our experimental findings may to some extent be used in the discussion of the question of the possible influence of the electromagnetic SHF field (many repeated exposures to fields of intensities higher than the permissible levels) on women, especially during pregnancy, and also on the course of the pregnancy and on the fetus. These findings may also be of practical importance in obstetric practice for the development of the necessary prophylactic and therapeutic measures.

* Many of the mice of this series were sacrificed so that special investigations could be carried out (histological examination, weighing of the ovaries and the uterine cornua). The fetuses which survived were investigated biologically (by the reaction to chorionic gonadotropin).

SUMMARY

As evidenced, there is a delay in the development and in the weight gain of the female mice progeny after subjecting them to the action of high-frequency electromagnetic field, during pregnancy. There were many stillbirths and deaths during the 3rd week of life among the progeny of these mice. The weight of the ovaries and of the uterine cornua was reduced in experimental animals. Mild histological changes in the ovaries and uterine cornua are not constant and occur mainly during the greatest functional activity of these organs—at the stage of rut.

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REGENERATION OF SKELETAL MUSCLE TISSUE OF WHITE MICE AFTER INJURY BY THE ELECTRIC CURRENT

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Research during recent years has demonstrated the high regenerative capacity of the skeletal muscle tissue of a number of mammals [5, 6]. The influence of factors of the external and internal environments on the course of the regeneration of skeletal muscle tissue has been studied [3, 4]. In spite of the high regenerative capacity of muscle fibers, muscle as a whole usually fails to regain its normal structure after injury. Khlopin [7], who investigated tissue cultures, concluded that one of the factors which prevented the complete recovery of muscle fibers is the abundant development of connective tissue in the wound. This was subsequently shown in experiments on muscle regeneration in the intact animal [6].

The object of the present research was to study the factors influencing the relative proportions of muscle fibers and connective tissue in the wound and, in particular, to ascertain how this reaction was related to the specific features of the injury inflicted.

EXPERIMENTAL METHOD

Investigations were carried out on sexually mature male white mice aged from 3 to 3½ months. The gastrocnemius muscle was injured in aseptic conditions by means of an alternating electric current of 127 v. Electrodes were placed directly on to the exposed muscle at its distal and proximal ends. Trauma was inflicted in the form of two or three electric shocks (the current was passed for 0.4-0.6 seconds). A 100 W lamp was connected in series in the circuit. The edges of the skin wound were kept clear of the electrodes. After trauma had been applied, the skin edges were approximated by one or two sutures. The operations were performed during the autumn and winter. The animals were kept at room temperature on an ordinary diet. The material for histological examination was fixed with Zenker's formol or neutral formalin at various stages from 10-15 minutes to 25 days after injury. Serial paraffin-wax sections were stained with hematoxylin-eosin, iron-hematoxylin, azure II-eosin, and by Mallory's method.

EXPERIMENTAL RESULTS

From 10 to 15 minutes after trauma, gross destruction of the muscle was found. Many muscle fibers were strongly contracted. This contraction was accompanied by irreversible changes in the myofibrillar apparatus, and led to amyloid degeneration of this apparatus. Some muscle fibers were ruptured in many places. Most of these tears were within the sarcolemma, i. e. they involved mainly the myofibrillary apparatus. The changes in the middle part of the muscle fibers between the points of application of the electrodes showed an exceptionally marked mosaic pattern. Most fibers were speckled with numerous waves of persistent contraction of the myofibrillary apparatus, of various shapes. In several cases the longitudinal fibrillary structure and the cross striation of the muscle fibers were replaced by a chaotic distribution of the myofibrils. In the deep layers of the wound

the muscle fibers showed granular degeneration, with the formation of masses of large granules, which sometimes escaped through the tears in the sarcolemma during contraction of the muscle and were situated between the muscle fibers. In other fibers granules were found in the form of chains along the individual myofibrils. In these cases it was possible to show that the granules were products of the disintegration of the myofibrillary apparatus. In certain muscle fibers the cross striation had almost disappeared. The disks of individual myofibrils were displaced in relation to each other, and their contours became merged. Such fibers subsequently underwent disintegration into large and small granules. At the points of application of the electrodes at the distal and proximal ends of the wound, as a result of the thermal action of the electric current the muscle fibers showed signs of necrosis, with the formation of necrotic masses very resistant to phagocytosis and autolysis. Besides the forms of destruction of muscle fibers described, various transitional forms were encountered. In this respect our observations were in agreement with the findings of Dmitriena [1], who investigated the relationship between various forms of destruction of muscle.

Fig. 1. Necrosis of traumatized muscle fibers 24 hours after injury. Stained with iron-hematoxylin. Magnification: objective 8 x, ocular 15 x.

One hour after injury, the pattern of necrosis of the muscle was essentially altered. Within the sarcolemmic tubes that remained, separate masses of the substance of the muscle fibers could be seen, in the form of disks, spirals, and so on. The spaces between the masses were filled with small amounts of finely granular material, resulting from the autolytic destruction of the muscle substance between areas of contracture of the myofibrillar apparatus. At this stage and later, until three days after trauma, many muscle fibers underwent gradually developing, finely granular degeneration. The granular mass thus formed differed, however, in its staining properties and the polymorphism of its granules, from the granular pattern described 10-15 minutes after injury. The source of the granules in this case was both the myofibrillary apparatus and the sarcoplasm. Subsequent changes in the necrotic masses took the form of swelling, crumbling, and conversion into detritus. The general picture of the degenerative phenomena on the first day after trauma was characterized by disintegration into large and small granules, with the arrangement of the necrotic masses in rows along the course of the former muscle fibers (Fig. 1). Among the necrotic muscle fibers, and within the fibers themselves, phagocytes were seen, although on the first and second, and even on the third day they were relatively few in number, and the disposal of the products of disintegration was mainly effected by autolytic processes. The spaces between the necrotic muscle fibers were permeated by connective tissue cells and invaded by capillaries containing blood. These surrounded the rows of granules and created a characteristic framework at the site of the disintegrating muscle fibers, thereby facilitating the preservation of the structural integrity of the pattern of the injured muscle (Fig. 2). On the fourth day the resorption of the necrotic masses in the greater part of the wound was almost complete.

The processes of regeneration in the muscle tissue developed on account of the surviving and partially injured muscle fibers in the deeper layers of the wound. Many muscle fibers, which preserved their normal structure,

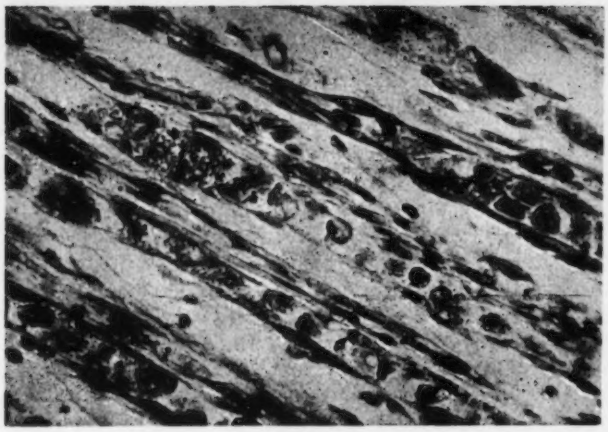

Fig. 2. Resorption of the necrotic masses and growth of the connective-tissue cells in the wound. Third day after trauma. Stained with azure II-eosin. Magnification: objective 40 ×, ocular 10 ×.

subsequently underwent partial disintegration or considerable reorganization. These fibers were the main source of regeneration of the muscle. The initial signs of regeneration were reasonably uniform, and were characterized by the accumulation of a basophilic sarcoplasm around the muscle nuclei. Spindle-shaped mono- and multinucleated sarcompasmic complexes were formed, which fused together along the length of the fiber and laid the foundations of the basophilic nucleated sarcoplasmic bands, occupying both a central and a peripheral position in relation to the axis of the muscle fiber. In the more severely damaged muscle fibers this process did not go far, for signs of degeneration developed rapidly in these fibers, affecting the old myofibrillary apparatus in the first place. Some of the multinucleated sarcoplasmic complexes which remained after destruction of the muscle fiber continued to develop into myosymplasts, and mitotic division of the nuclei sometimes took place in these structures.

In the less severely injured muscle fibers, the process of formation of the nucleated sarcoplasmic bands went further, and a large amount of sarcomplasm accumulated and the nuclei divided intensively. There could be several such bands in a muscle fiber. Those which lay immediately beneath the sarcolemma could become detached from the muscle fiber and become converted into free myosymplasts. When several nucleated sarcoplasmic bands were present in the muscle fiber, the latter could break up at once into a whole bundle of myosymplasts. After strong stimulation with the electric current many muscle fibers, although remaining outwardly little changed, could not exist for long without reorganization of the whole internal system, so that their differentiation and conversion into structures of the myosymplast type, capable of development, must be regarded as progressive phenomena.

The splitting of muscle fibers in the depth of the wound was also important for the filling up of the more superficial layers of the wound defect with myosymplasts. Here, however, we often found fine myosymplasts, accompanied by spindle-cells with basophilic cytoplasm, growing along the rows of necrotic masses. From the investigation of the relationship between these cells and the myosymplasts mentioned above, it may be supposed that after injury to a muscle by the electric current, the myoblasts play some part in regeneration.

A characteristic feature of the regeneration of muscle after injury by the electric current was that in almost every case the myosymplasts grew parallel to each other. To a large extent this could be explained by the splitting of the myosymplasts from the old muscle fibers in the depth of the wound. The principal cause of the favorable course of regeneration, however, was absence of a wound cavity and of mechanical displacement of the tissues as a result of the trauma, and also the partial preservation of the fibrous structures of the endomysium. A new connective-tissue framework, with myosymplasts growing into it, was formed from the remnants of the old framework. Favorable conditions of tension were created in the wound, and this encouraged regeneration of the muscle [6]. The myosymplasts as a rule grew between the dying fibers and did not utilize the old sarcolemmic

Fig. 3. Differentiation of young muscle fibers. Twelfth day after trauma. Stained with iron-hematoxylin. Magnification: objective 40 ×, ocular 7 ×.

sheaths, as was described by Zenker [8], who investigated the regeneration of muscles in typhoid fever. A certain part of the fibrous structures of the old sarcolemmas evidently entered into the composition of the connective-tissue foundation of the new muscle, but on the whole the sarcolemmas of the regenerating muscle fibers were formed anew.

In most cases by the end of the fourth day and on the fifth day after trauma a considerable number of myosymplasts had accumulated in the wound. Their orderly arrangement facilitated their rapid growth and differentiation. On the sixth day a myofibrillary apparatus had formed in the myosymplasts, and these were converted into muscle tubes. Growth and differentiation of the latter also took place quickly. The number of their nuclei diminished, and on the 10th-12th day young muscle fibers were formed (Fig. 3). On the 15th day they had attained a considerable thickness, and only the presence of small areas with an incorrect arrangement of fibers indicated that we were dealing not with old muscle fibers undergoing reactive changes, but with young tissue. On the 25th day the number of nuclei in the muscle fibers was very small; most nuclei were situated beneath the sarcolemma, and fibers were rarely found with short chains of nuclei. The general character of the arrangement of the muscle fibers had become much nearer to normal.

Regeneration of the muscle was complicated only by the presence of peculiar necrotic masses resistant to both phagocytosis and autolysis. These were found in the distal and proximal parts of the wound, at the points of application of the electrodes. Their number was related to the degree of the thermal action of the current. These necrotic masses were only destroyed by the 10th-12th day, when myosymplasts from the middle parts of the wound enveloped them. The differentiation of the muscle fibers in this region was considerably delayed. Nevertheless, the main property of the regenerating muscle fibers—their orderly arrangement—remained unchanged. Although our observations ended on the 25th day of the course of regeneration, there is, therefore, every reason to suppose that subsequently the regenerated muscle remained a perfect structure, in both morphological and physiological respects.

Although injury by the electric current was accompanied by severe destructive processes in the muscle, its normal structure was thus almost completely restored. One of the more important reasons for this was the presence of a supporting framework, directing the growth and movement of the connective-tissue elements and the myosymplasts.

SUMMARY

Regeneration of gastrocnemius muscle was studied in white mice after an injury inflicted by electric current (127 v.). Electric lesions of the muscle result in complete destruction of a considerable number of its fibers. Degeneration of the muscle fibers is varied and mosaic in character. Small lump, large lump, and granu-

lar forms of degeneration are prevalent. The absence of wound cavity, rapid resorption of necrotic masses, preservation of some fiber structures in the injured muscle endomysium create favorable conditions for regular growth and free movement of young connective tissue and myosymplasts. In such conditions connective tissue does not prevent regeneration of the muscle, the normal structure of which is restored almost completely.

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UNIVERSAL STEREOTAXIC APPARATUS DB-2-59 (SECOND MODEL)

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Translated from Byulleten' Éksperimental' noi Biologii i Meditsiny, Vol. 51, No. 5, pp. 113-115,
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In 1957 we designed and constructed the stereotaxic apparatus DB-1-57 [1]. The present model (see figure) is considerably improved and is intended for neurophysiological and neuromorphological investigations in the region of the brain and spinal cord of cats, rabbits, and small monkeys.

BRIEF DESCRIPTION OF CONSTRUCTION

The main bearing frame (1) of the apparatus, as distinct from the first model, is also a rotating frame and consists of two longitudinal square steel rails parallel to one another. At the rear end the rails are cylindrical. The ends of the rails are fixed by screws in holes in two transverse triangular plates (2), thus forming a rigid frame. The frame can rotate through 360° on two half-axles (3) inserted in the holes of the L-shaped stands (4), which form the support of the apparatus. The two stands are bolted on to a laboratory bench.

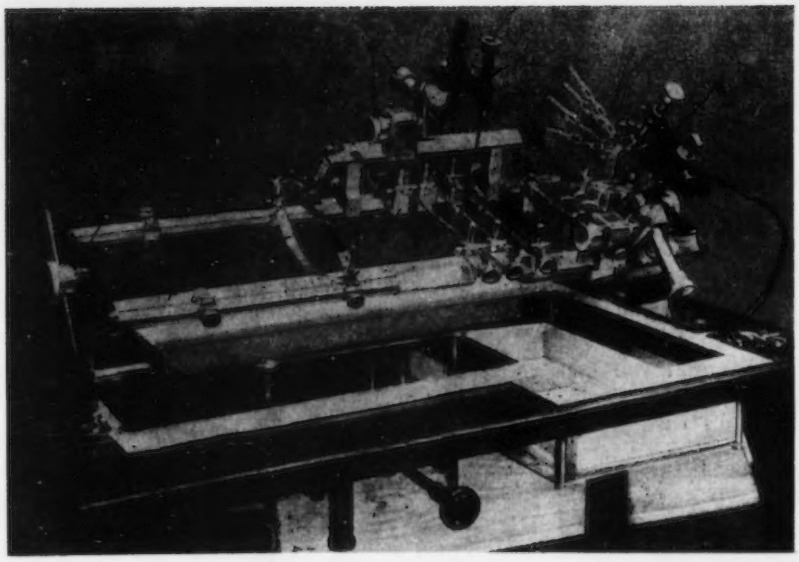
Thus, the main portion of the apparatus consists of two L-shaped stands and a frame rotating on two half-axles. The experimenter can fix the frame in any desired position by tightening the retaining nuts on the half-axles. Thus, the apparatus allows rotation of the secured animal with the inserted brain electrodes and micromanipulators attached to the apparatus, and permits easy access to different parts of the animal's body.

The known models of stereotaxic instruments [2-11] do not provide such an opportunity. An exception is Delia's modification, but the absence of a second pivot in that apparatus makes rotation practically impossible. We have become convinced of this by more than two years' experience of working with that model.

For the attachment of the interchangeable head-holders there is a special II-shaped frame (5) which allows rotation of the head-holder round a main axis passing through the external auditory passages of the animal. As distinct from the first model, the motion is effected by a skew-toothed circular rack driven by rotation of a pinion. The segment is graduated and allows the animal's head to be tilted upward to 45° and downward to 55°. The frame of the head-holder is rigidly connected to the rack and can be fixed in any of the above positions. The transverse stage of the frame carries the removable head-holder and also posts for the attachment of small appliances (electrode distributing panel, flexible electrode-holders, etc.).

The head-holder for a cat consists of a rectangular stage with a lengthwise groove for the fixing nut. Two orbital fixers, which can easily be adjusted to the size of the skull, are screwed to the top of the stage. The stem of the triangular frame for fixing the upper jaw is also attached here by a side screw.

The auditory passage fixers are cylindrical and are furnished with millimeter divisions for setting the animal's head on the center line of the apparatus. For adjustment of the electrode to the vertical zero position the auditory passage fixers are fitted with calibrated rings of radius 10 mm (corresponding to the zero horizontal of Horsley and Clarke). There are marks on the rings for determination of the zero frontal plane. This important feature is absent in other models of stereotaxic instruments. In R. M. Meshcherskii's model [2] an independent post is used for fixing the position of the zero horizontal.



Universal stereotaxic apparatus DB-2-59 as most generally seen. Explanation in text.

In works on rabbits another head-holder is used (not shown in figure). The head-holder assembly includes a rectangular plate with a recess for the cutters and capable of travel in the anteroposterior and vertical directions, two brackets with orbital and jaw fixers, the brackets being attached to the side arms of the II-shaped frame of the head-holder in place of the auditory passage fixers for the cat. In work on small monkeys (rhesus) it is sufficient to replace the orbital fixers of the cat head-holder.

This model, as distinct from others, is provided with metal straps (6) embracing the animal's body above and below and preventing sagging of the body when the frame of the apparatus is rotated. The straps are attached to two pairs of brackets, which can be moved along the rails of the apparatus. There is also a pair of brackets for securing the hind limbs (7).

During work on the spinal cord the spine is held by steel spokes 2 mm in diam. which are fitted with tube stops (8), the heads of which bear on the spine and restrict lateral movements. The spokes with the tube stops are held in special sockets, which in turn are attached to sliding brackets (9). The brackets can travel along the rails and can be fixed in the required position.

In another arrangement the sockets holding the spokes are attached to special rails lying alongside the main rails of the apparatus. These extra rails are fastened to the same brackets, but the latter are placed far apart. In the first case the micromanipulator travels along the spinal cord on a special rail fastened parallel to the apparatus frame by two brackets. In the second case the micromanipulator travels along the main rail. The second arrangement is better, but both methods ensure rigid fixation and effective operation with the microelectrodes.

This model differs from all other known models in possessing a hot table (10) consisting of a rectangular brass box (screen) covered on top with a sheet of transparent plastic. Inside the box are an electric heater (127 v) and a contact thermometer with a controlling magnetic head to which a direct current of 24 v is supplied. The box is grounded. The power is fed through a screened cable. The controlling relay is located outside the chamber. There is practically no interference. The system ensures automatic maintenance of the preset temperature. The hot table is provided with a lifting mechanism and is mounted in a separate frame attached to the bench by cap screws. For turning the animal the heated stage must be lowered and then raised again after the animal is turned.

Micromanipulators. The assembly includes two micromanipulators (11) which provide coarse and fine adjustment of the electrode to within 5 μ in three mutually perpendicular planes. The coarse adjustment is effected as follows: in the frontal plane—by sliding the main bracket of the manipulator along the rail of the

instrument; in the sagittal plane – by sliding the bushing in the bracket; in the horizontal plane – by the movement of the rack connected to the vertical arm of the micromanipulator. The fine adjustment is provided by the rotation of verniers (12), the circumference of which is graduated in 100 divisions.

The vertical arm of the manipulator can be tilted through 90° left and right in the sagittal plane and through 45° in the frontal plane. Thus, the design of the manipulators allows operation in a system of rectangular co-ordinates or in a system of tangential (equatorial) co-ordinates. This ensures much greater accessibility to any structures of the brain.

The assembly of the apparatus includes eight flexible hinged electrode-holders (13) attached by a special shoe to the posts on the head-holder or to the brackets of the spine fixer. Before the electrode is inserted by the manipulator, the thick end of the former is fitted into the split socket of the electrode-holder. When the electrode has been inserted into the appropriate brain structure, the split socket of the micromanipulator is released and the vertical arm is raised. The electrode is held in place by the flexible electrode-holder. Hence, several electrodes (up to eight) can be inserted by the one micromanipulator, and placed fairly close together. This feature is not provided by any other model, and yet it greatly enlarges the range of investigations.

Thus, the second model of the stereotaxic apparatus allows simultaneous work in the region of the brain and spinal cord of laboratory animals (cats, rabbits, monkeys). It has a device for holding the spine in position and allows rotation of the frame together with the secured animal, micromanipulators, flexible electrode-holders, and inserted brain electrodes. The design of the micromanipulators permits operation in a system of rectangular or tangential co-ordinates. There is a device for tilting the head and a set of interchangeable head-holder parts. The apparatus is provided with a device for fixing the body and limbs of the animal and a hot table with lifting mechanism.

These design features of the described model, which distinguish it from all other known instruments, considerably extend the range of investigations and justify its being called a universal stereotaxic apparatus.

SUMMARY

The authors describe a new model of a universal stereotaxic instrument, intended for the neurophysiological and neuromorphological investigations in the area of the brain and spinal cord of cats, rabbits, and small monkeys. The instrument is supplied with two micromanipulators operating in the system of rectangular and equatorial co-ordinates. The stand of the device is capable of being turned around 360°, together with the fixated animal and inserted electrodes. The head-holder may be tilted. The instrument is provided with an automatic heating device and an adjustment for the fixation of the spinal column.

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A NEW MODEL OF STEREOTAXIC APPARATUS FOR SMALL LABORATORY ANIMALS

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Stereotaxic apparatus has been widely used during the past 30 years. There are a great number of descriptions of such apparatus, differing in mechanism and adapted for different animals [1, 4], in foreign literature. Recently, a report appeared in our literature with descriptions of the original constructions. R. A. Durinyan and A. I. Bartyzel' [2] described a model of stereotaxic apparatus designed for operation on cats. An apparatus described by R. M. Meshcherskii [3] provides the possibility of working with animals of varying sizes. Its mechanism is rather complex and can be manufactured only under factory conditions.

In research using methods of electrolytic injury of various sections of the brain the need often arises to perform operations rapidly on a large number of animals. This necessitates a stereotaxic instrument. It must be simple and convenient to use, but at the same time must assure a rapid and reliable stereotype fixation of the animal's head and sufficient precision in the placement of the electrodes within the required boundaries. Moreover, in the construction of stereotaxic apparatus the preparation of the electrode placement mechanism, which assures the required precision of localization of injuries produced, often presents difficulties. We published an account of a simple model of stereotaxic apparatus designed to work on rats [1], in which the mechanism of electrode placement consisted of two specimen carriers* ST-12. A test of this model in our laboratory in 1958-59 showed that the use of such a device as the electrode placement mechanism, assuring the precision of their placement to 0.1 mm, is quite satisfactory for producing precise localized injuries (diameter to 0.3-0.5 mm) in the deep-lying sections of the brain. At the same time, the use of specimen carriers facilitates the preparation of a stereotaxic instrument and assures simplicity in working with it.

In the present article a description of a more improved and more convenient to operate model of stereotaxic apparatus is presented, in which the specimen carrier ST-12 is also used. A schematic picture of the apparatus is presented in Fig. 1.

The apparatus is mounted on a panel (1), manufactured from 10-millimeter textolite or ebonite (for rats, suitable panel dimensions are 200 x 300 mm). Fixation of the head is accomplished by ear and tooth holders. The two ear holders (2) are made of ebonite (in Fig. 1 the left ear holder is not pictured; only its support is shown). For rapid fixation of the operated animal's head the ear holders are freely shifted in the metallic supports (3); their movement is accomplished by paired springs (4), the tension of which can be regulated by the movement of the screw-threaded washer (5) at the metallic terminal of the ear holder (6). For a rigid fixation the ear holders, after their insertion into the acoustic ducts of the skull, are tightened by screws (7).

* The devices used are apparently modified from the driving mechanisms of calibrated microscope stages. – Publisher.

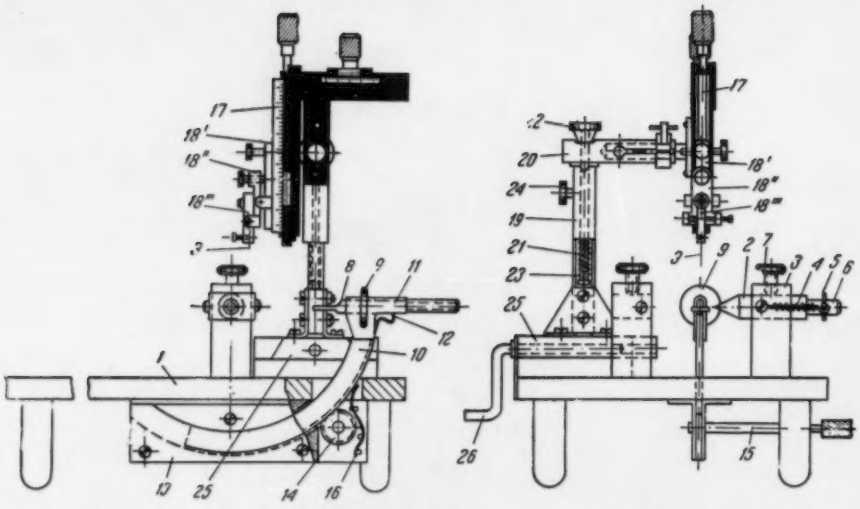

Fig. 1. Schematic picture of the apparatus. 1) Panel; 2) ear holder; 3) ear holder support; 4) ear holder spring; 5) washer; 6) terminal of the ear holder; 7) clamp screw of the ear holder; 8) teeth holder; 9) nut controlling the ear holder; 10) sector (rack); 11) brace; 12) tooth holder spring; 13) stock; 14) gear wheel (pinion); 15) gear wheel axis; 16) sector spring; 17) carrier ST-12; 18', 18", 18'''—3 articulation knobs; 19) holder support; 20) arm holder; 21) pivot; 22) pivot nut; 23) holder spring; 24) holder clamp screw; 25) transverse shifting mechanism; 26) screw for mechanism.

The tooth holder (8) is a metallic pivot with a screw thread, the end of which is compressed and has an opening into which the animal's incisors are inserted. The tooth holder is shifted in a forward-backward direction by means of turning a special nut (9).

In the present model there is provision for change in the angle of inclination of the head of the operated animal in a sagittal plane, which is required in the use of different topographical maps of the brain. This is accomplished by movement of the sector (10), which is graduated in angular degrees. The tooth holder is fastened to the sector by means of a brace (11). A spring (12) is used in order to allow clearance of the tooth holder. The sector is located in the stock (13), consisting of two plates adjacent to the sector at top and bottom, and two angular lateral walls by which the stock fastens to the panel. In the forward part of the stock a gear wheel (14) is mounted with an extended axis (15), the rotation of which is accomplished by movement of the sector. A spring (16) was used in order to allow for clearance of the sector in the stock.

The carrier ST-12 (17), fastened vertically, was used as the mechanism of electrode placement. The electrode (E), made of plexiglass and consisting of 3 components (18', 18", and 18'''), is inserted into the head. A knob permits orientation of the vertically set electrode, since it is possible to change the angle of orientation of the electrode in sagittal and frontal planes. With the aid of a specimen carrier a sagittal and vertical shift of the electrode is accomplished. It secures to a holder, enabling one to draw aside the carrier from its working position. A spring (23), attached to the pivot and placed in a cut in the support, provides fixation of the arm of the holder in a working position. A clamp screw (24) serves for rigid fixation in this position. The weight of the holder supporting the specimen carrier facilitates (with the aid of anglebars) the transverse shifting mechanism (25), which is made of ebonite in the shape of a swallowtail, the movement of which is achieved by a screw with a handle.

Working with the Apparatus. Before carrying out the operation, electrodes were inserted into the head, which then was oriented vertically in sagittal and frontal planes. Our experiment shows that vertical insertion of an electrode "by eye" was quite possible with the use of the usual metal anglebar as a standard. For convenience, the ear holder can be slid and fastened in position by corresponding screws. After setting the electrode, the screw securing the pivot holder is loosened and the arm of the holder is slightly raised by hand and is turned aside. Then, the head of the operated, previously narcotized animal is fixated by the ear holders in the auditory ducts (Fig. 2). For convenience, one holder is left fastened to the support; the clamp screw of the other is loosened.

With one hand the experimenter holds the head of the animal and with the other turns the movable ear holder. At first, the auditory duct is brought to the immovable ear holder; then, the movable one is softly inserted in

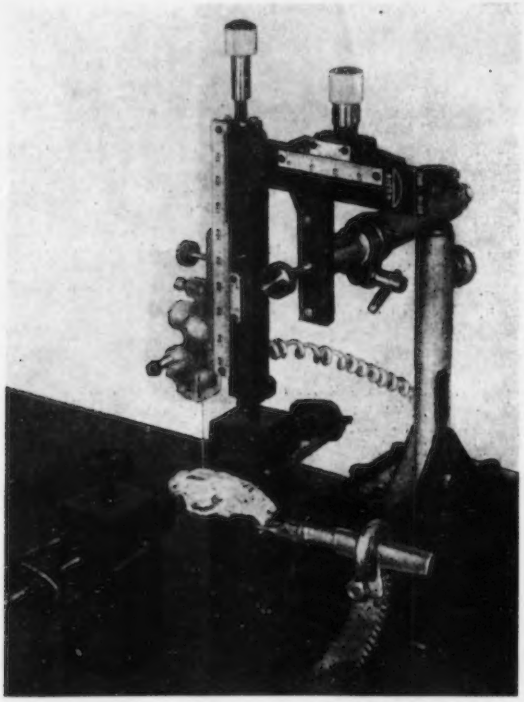

Fig. 2. General view of the apparatus.

the other auditory duct. The screw fastening the immovable ear holder is loosened, and under the effect of the spring the animal's head is suspended in the middle between the supports. Turning the head in a sagittal plane, the incisors are inserted in the corresponding opening of the tooth holder, which then, by the turning of its nut, is retracted for a tight fixation of the animal's head, making it possible to control any tremor. The ear holders are fastened by screws. Turning the sector sets a low angle of inclination of the head, after which one can proceed with the operation.

SUMMARY

A stereotaxic device simple in construction and operation is described. It allows achieving local electrolytic injuries of deeply located cerebral portions in small laboratory animals.

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CHAMBER FOR OBTAINING AUTOGRAMS OF ORGANS

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In 1954 N. N. Belavina [1, 2, 3] suggested the method of autography for obtaining the image of the surface of an organ or slice of organ on a film by direct contact with the emulsion layer of the latter. Images of organs by direct contact with a film have also been obtained by other research workers [4].

In the autographic procedure the organ under investigation is carefully laid on a glass plate. A film is then laid on top of the organ in the dark, and pressed down by another glass plate. The plates are then turned through 180° so that the film is underneath and the organ lies on its emulsion layer. The organ with the film and plates is then wrapped up in black paper and placed in a refrigerator for exposure.

One of the faults of this method is that when the film is laid on the organ and the glass plates are turned over the film becomes warped and the organ is often displaced, thus resulting in the obtaining of blurred autograms. Moreover, the organs are laid on the film in the darkness, and hence there is no guarantee that the organ is placed in the center of the film and that an autogram of the whole surface of the organ will be obtained. Finally, it is difficult to obtain in identical conditions autograms of organs of control and experimental animals.

In order to facilitate and improve the method of obtaining autograms, we constructed and tested a chamber which, in our view, is the answer to the problem (Fig. 1).

The main material for construction of the chamber is 3 mm thick textolite sheet. The chamber consists of a box with grooves in the top into which two 9 × 12 cm film holders from the "Fotokor" camera fit freely and closely. On the floor of the chamber there is mounted a post, which can be raised or lowered by means of a worm screw (setting mechanism), driven by a knob on the outside. Attached to the knob is a pointer, which indicates the extent of the vertical motion of the post.

Rigidly attached to the top of the post and parallel to the plane of the film holders is a stage carrying another two 9 × 12 cm stages mounted independently of one another on flexible flat springs directly below the film holders. To the top of these stages is cemented a layer of soft sponge rubber 1 cm thick. The independently adjustable mounting of the stages and the presence of the sponge rubber layer permits the simultaneous autography of organs of different size. The sponge rubber presses the organ or slice against the surface of the film and prevents the displacement or sliding of the organ on the film.

The organs or slices are laid on the movable stages through the openings in which the film holders are inserted. The whole system is lowered to the bottom of the chamber by means of the knob (dial), and then the loaded film holders are inserted. The covers of the holders are removed and the organs are raised to make full contact with the film by means of the dial. In this position the chamber is left in the refrigerator for exposure. At the end of exposure the stages carrying the organs are lowered. The film, which becomes tightly stuck to the

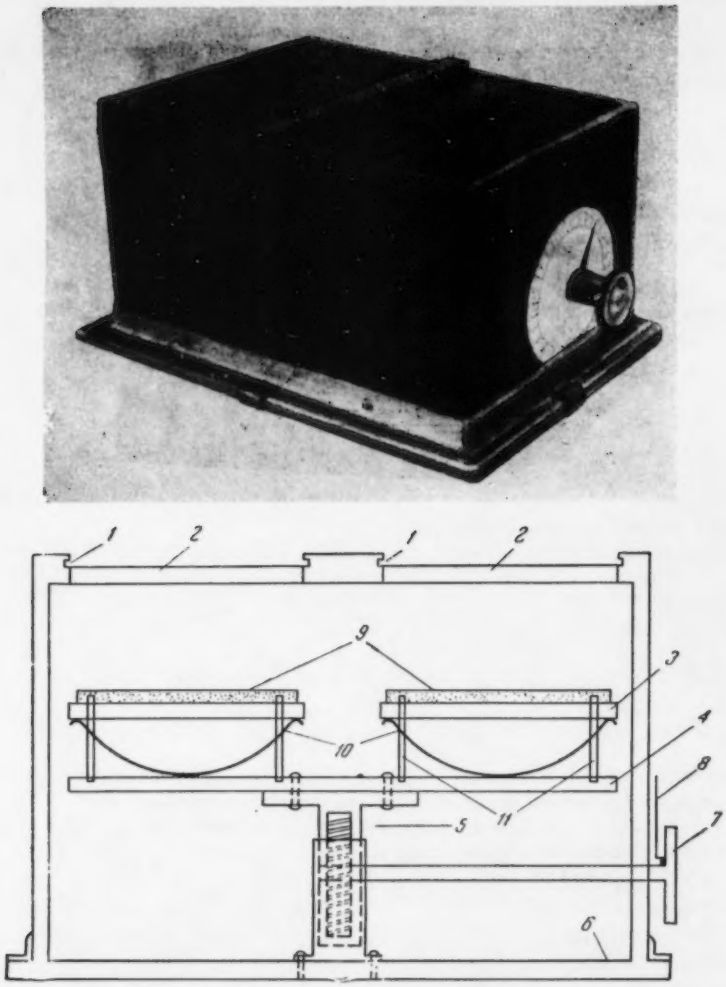

Fig. 1. General view and schematic diagram of chamber for obtaining autograms. 1) Grooves for film holders; 2) openings for film holders; 3) movable stage; 4) common movable stage; 5) adjustable post; 6) detachable bottom of chamber; 7) knob of setting mechanism; 8) pointer indicating height of lift; 9) rubber cushion for organs; 10) springs; 11) guide posts.

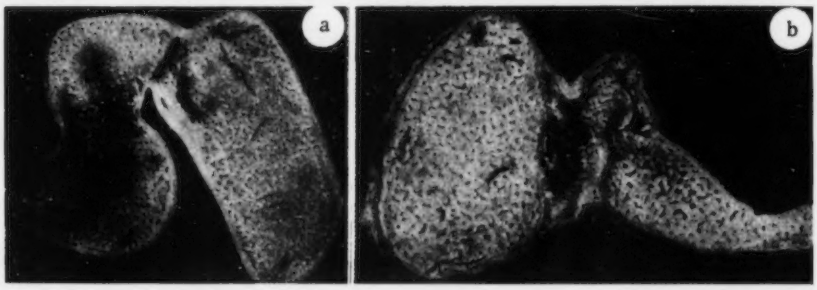

Fig. 2. Rat liver autograms obtained with chamber after 24 hr exposure: a) Surface of liver of normal rat (control); b) surface of regenerating liver four days after removal of two liver lobes by method of Higgins and Anderson.

organs during exposure, comes down at the same time. The holders are taken out in the dark room, and the film and organs are carefully removed from the stages and immersed in water for separation. The subsequent procedure for obtaining the autograms (development of film, etc.) is as usual.

The autograph chamber described enables the obtaining of clear autograms (Fig. 2) and allows most of the procedure to be conducted in the light under visual control. The chamber is of such simple construction that any research worker can make it.

SUMMARY

The author describes an original autographic chamber for obtaining imprints of the organs on a film. Autograms of rat liver obtained with the aid of this chamber are presented.

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ELECTRONIC LOW-FREQUENCY ANALYZER AND ITS USE IN ELECTRORETINOGRAPHY

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Accurate frequency analysis is an important problem in electroretinographic and electroencephalographic studies.

A determination of the stimulation rhythms which can still be reproduced by the particular system is of special importance. This ability, as the classical studies of N. E. Vvedenskii showed, characterizes the functional mobility (lability) of the system under investigation.

The ability of the visual analyzer to reproduce a rhythm of photic stimulation has been the subject of numerous investigations, but no one has yet determined exactly what links in the visual organ system govern this ability, which is manifested psychophysiologically in the function of the flicker fusion frequency. The retina is the first link involved in the accomplishment of this function. Until recently the ability of the retina to reproduce a rhythm of photic stimulation was assessed from the electroretinogram obtained in response to flickering light. However, it was difficult to settle the question of the maximum photic rhythm reproducible by the retina since an increase in the flicker frequency led to a considerable reduction in the amplitude of the responses on the electroretinogram and the amplifier noise masked the induced fluctuations of electric potential. The maximum reproduction of a photic rhythm in these conditions did not appear to exceed 50-55 flashes per sec. Henkes et al. [1], who used an electronic frequency analyzer for clarification of this question, succeeded in showing that the human eye could reproduce a flicker frequency of up to 70-75 per sec, and in one case even up to 94 per sec.

Since the maximum flicker fusion frequency in man does not usually exceed 60 flashes per sec, we can infer that the retina is not the link in the visual analyzer system which normally determines the fusion frequency.

Led by V. K. Zhdanov, we constructed in our laboratory an electronic low-frequency analyzer capable of detecting bioelectrical potentials commensurable with the noise level.

The technical data of the instrument were: amplification factor 30, frequency range 8-130 cps, minimum passband at level 0.7 not broader than 4.5% of the frequency of the investigated signal. The instrument can be connected up in an arrangement with a symmetrical and asymmetrical input and output. The operating principle of the selective amplifier was based on the use of negative feed back effected through a twin-T bridge (Fig. 1, a).

In our experiments the instrument was connected between the third and fourth stages of one of the channels of a standard 15-channel electroencephalograph (Alvar firm). A block diagram of the connection is shown in Fig. 1, b.

With this instrument, as distinct from a number of other types of analyzers used in modern electroencephalography, the record of periodic processes on film or on paper does not consist of code marks indicating the

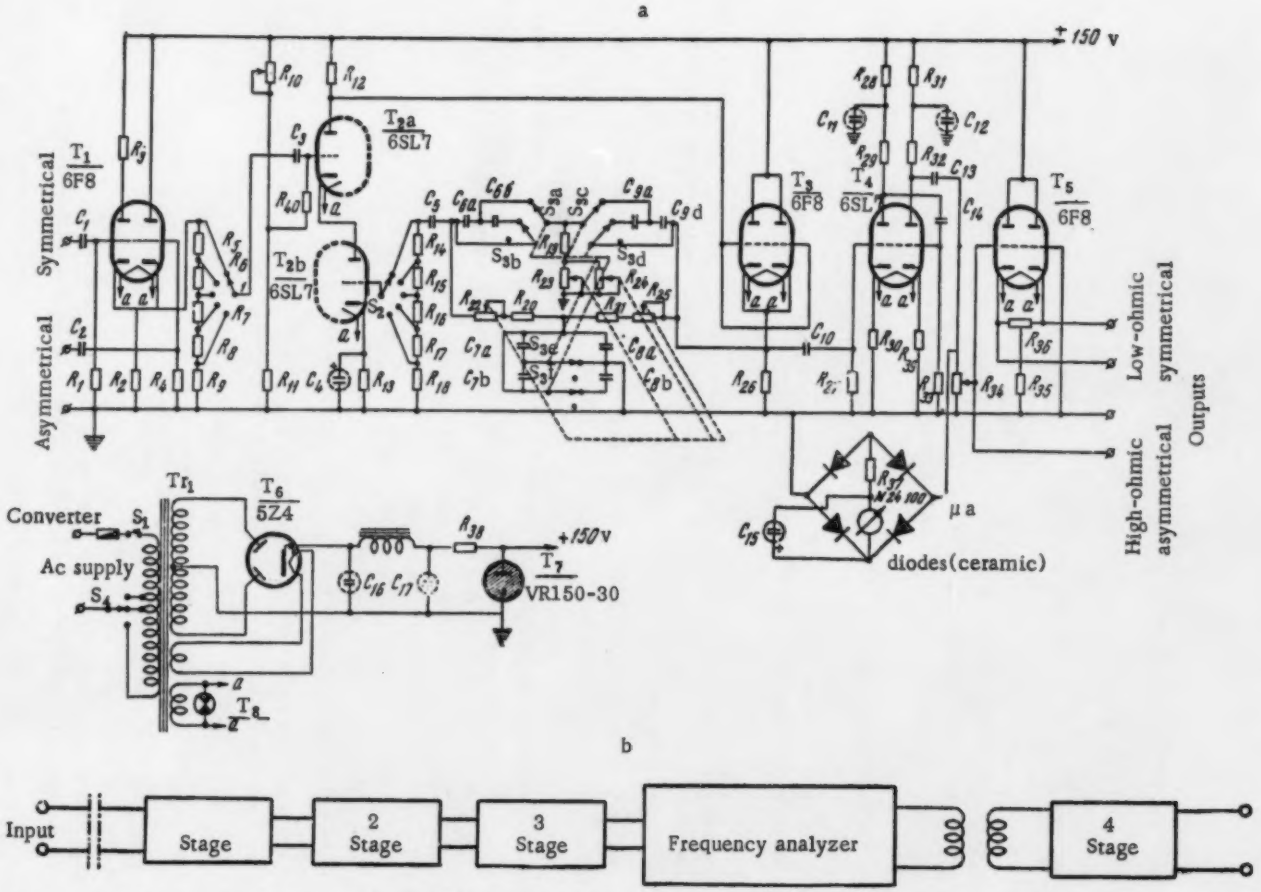


Fig. 1. Low-frequency analyzer. a) Circuit of amplifier of low-frequency analyzer; b) block diagram of connection of analyzer in amplifier channel.

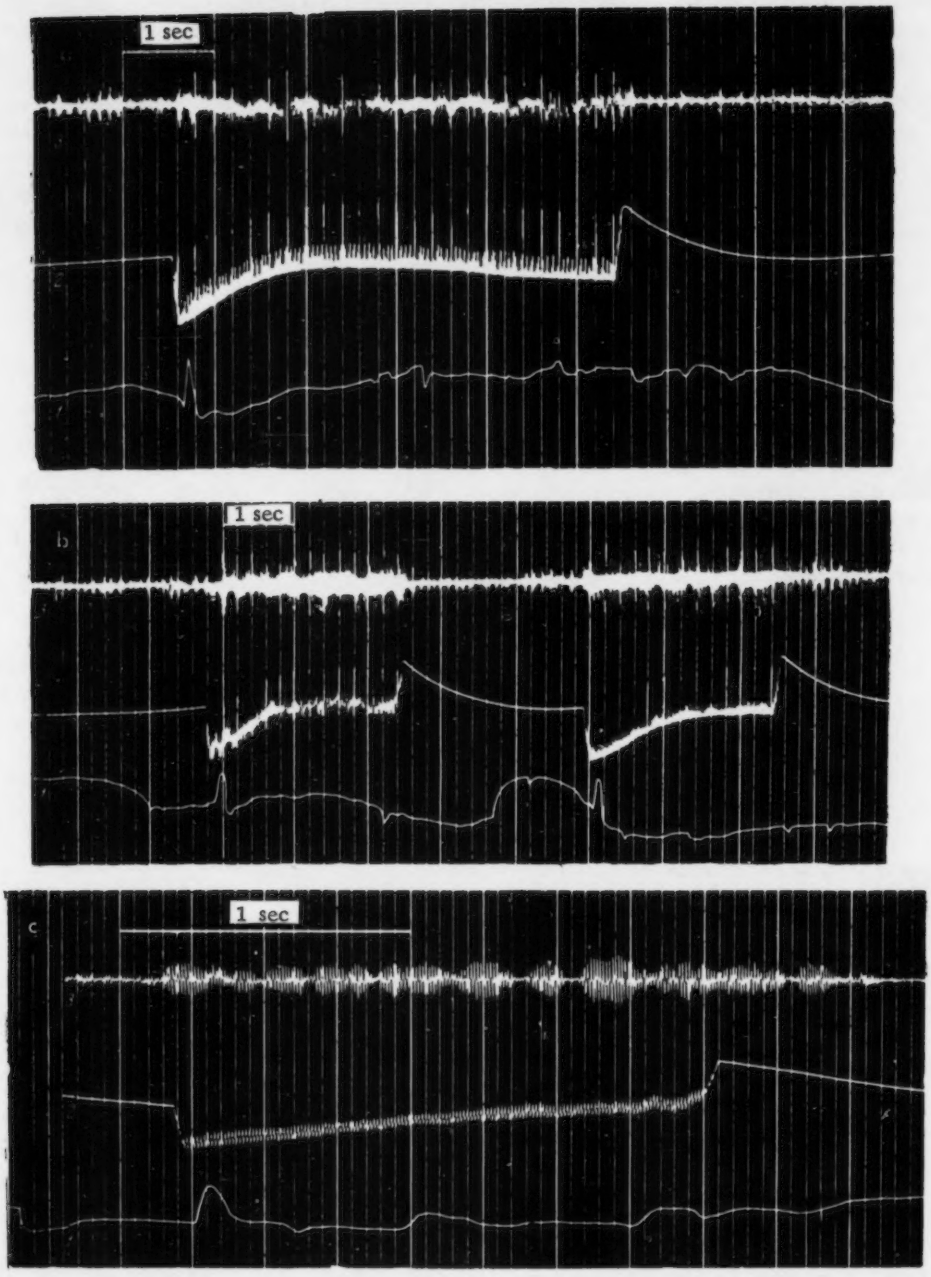


Fig. 2. Analysis of frequencies reproduced by human retina on stimulation by interrupted light. a: 1) Electroretinogram; 2) stimulation marks, 40 cps; 3) frequency analyzer reveals frequency of 40 cps reproduced by retina, but indetectable on electroretinogram; b) as above, analyzer reveals frequency 60 cps reproduced twice by retina; c) as above, analyzer reveals frequency of 100 cps reproduced by retina with after-effect of about 0.5 sec duration.

presence and amplitude of a component of particular frequency, but is a direct reproduction of the response to a rhythm of photic stimulation. This was made possible by the high selectivity of the amplifier. Narrowing of the passband enabled a considerable reduction in the noise level at the amplifier output. Low-capacity capacitors were connected at the channel input to eliminate the effect of slowly varying potentials.

Our observations were made on two normal human subjects. Our aim was to discover the maximum flicker frequency reproducible by the retina on the electroretinogram when our low-frequency analyzer was used.

The electroretinograms were recorded by means of a chlorided silver electrode mounted in a contact lens fitted on the eye. The space between the lens and eye was filled with physiological saline. The indifferent electrode was placed on the ear lobe. The subject was placed in a screened cabinet. Dark-adaptation took 5 to 10 min. For the photic stimulation we used a 500 w projection lamp, which gave an intensity of about 800 lux at the eye level, and a light chopper.

Our investigations showed that the normal human retina can reproduce a rhythm of up to 100 cps (Fig. 2). This cannot be established in the usual electroretinogram without the use of the analyzer.

We must mention that the reproduction of such a high flicker frequency in the electroretinogram was not obtained in every case, even in the same subject. For instance, one of our subjects, A. P., whose electroretinogram usually revealed the reproduction of a photic rhythm of up to 100 cps, could only reproduce a rhythm not exceeding 45 cps in one of the experiments. We learned that on the day before he had been engaged in electric welding for several hours and was very tired. This observation is in accordance with the established fact of a relationship between the electroretinogram and the general state of the organism.

SUMMARY

The electronic analyzer of low frequencies suggested by V. K. Zhdanov, may be successfully used for frequency analysis of electric biopotentials, particularly to analyze the electroretinogram. With the aid of this apparatus the authors recorded the reproduction of light rhythm in electroretinogram, equal to 100 cycles per sec.

The authors were the first to record such rhythms in humans.

LITERATURE CITED

1. H. E. Henkes et al., Ophthalmologica 132, 14 (1956) p. 140.•

• Omitted in original and inserted by translator.

APPARATUS FOR LYMPH NODE PERFUSION

Yu. I. Borodin

Department of Normal Anatomy of Novosibirsk Medical Institute
(Presented by Active Member AMN SSSR V. N. Ternovskii)
Translated from Byulleten' Éksperimental' noi Biologii i Meditsiny, Vol. 51, No. 5,
pp. 124-125,
Original article submitted June 6, 1960

In connection with the study of the numerous functions of the lymphatic system, an investigation of the carrying capacity of the lymph node (its transport function) in various physiological and pathological conditions is of great interest.

A valuable method of studying the functions of the lymph node is the perfusion of it with particular fluids. This method was used in the studies of Drinker and Yoffey [2], and also by V. N. Chernigovskii and his colleagues. Chernigovskii has made extensive use of this method in his investigations.

Our aim was to design a lymph node perfusion apparatus which could be used for a quantitative determination of the transport function of the lymph node.

For an assessment of the transport function of the lymph node the volume of liquid flowing into the node and out of it in unit time must be determined. Calculation of this volume merely requires an investigation of the rate of flow of the liquid in graduated glass tubes connected with the afferent and efferent lymph vessels. If the rate of travel of air bubbles in these tubes and the diameter of the tubes are known, it is easy to determine the volume of liquid passing through the tube in unit time into the node or out of the node. Changes in the flow into the node and out of it indicate the changes in the transport function of the node in the particular experimental conditions.

Proceeding from this principle, we set up a special apparatus for lymph node perfusion. This apparatus has been used for perfusion of the lymph nodes of dogs, cats, and rabbits in our investigations [1], and also in research by our colleagues in the Department of Normal Anatomy of Novosibirsk Medical Institute [3].

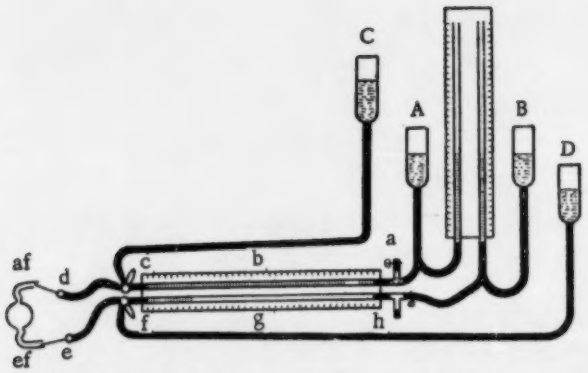
The arrangement of the apparatus is as follows (see Figure).

From an ampoule A mounted at a preset height, the perfusion liquid passes through a system of rubber tubes to the needle d, inserted in the afferent lymph vessel af.

The volume of liquid flowing into the node is determined from the rate of travel of an air bubble from right to left in the graduated glass tube b. As soon as the air bubble reaches the end of the scale, liquid from ampoule C, placed higher than ampoule A, is admitted into tube b by a turn of the three-way stopcock c. The air bubble is driven back to the start of the scale and observations can then continue. Thus, by manipulation of stopcock c the experiment can be continued for as long as necessary.

After passing through the lymph node the liquid (physiological saline in our experiments) flows through the needle e inserted in the efferent vessel ef and then into the graduated tube g. The air in the latter is displaced from left to right by the liquid. When the whole tube is filled the excess liquid can be run off into ampoule D by a turn of the three-way stopcock f. For this purpose ampoule D must be brought below the level of tube g.

The blind branches a and h serve for the admission or removal of air from the system, and when necessary they can be closed with clamps.



Apparatus for lymph node perfusion. Description in text.

The described system can also be used for the simultaneous perfusion of two lymph nodes. In this case the liquid from ampoule A is fed into one node, and that from ampoule B is fed into the other. In this case needles d and e are inserted in the afferent vessels and the efferent vessels are transected. With this experimental set-up only the volume of liquid flowing into the node can be determined.

The metal three-way stopcocks c and f were copied from Val'dman's apparatus for measurement of venous pressure.

SUMMARY

An apparatus was designed to investigate the transport function of lymph nodes in experimental conditions. This apparatus has made it possible to determine the volume of fluid entering and escaping from the lymph node per unit of time. Altered transport function of the node in various experimental conditions may be assessed by the changes in these values.

LITERATURE CITED

1. Yu. I. Borodin, in: Problems of Morphology [in Russian] (Novosibirsk, 1958) p. 107.
2. C. K. Drinker and J. M. Yoffey, Lymphatics, Lymph, and Lymphoid Tissue (Cambridge, 1941).

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

Soviet Journals Available in Cover-to-Cover Translation

ABBREVIATION	RUSSIAN TITLE	TITLE OF TRANSLATION	PUBLISHER	TRANSLATION BEGAN Vol.	Issue	Year
AÉ	Atomnaya énergiya	Soviet Journal of Atomic Energy	Consultants Bureau	1	1	1956
Akust. zh.	Akusticheskii zhurnal	Soviet Physics – Acoustics	American Institute of Physics	1	1	1955
	Antibiotiki	Antibiotics	Consultants Bureau	4	1	1959
Astr(on). zh(urn).	Astronomicheskii zhurnal	Soviet Astronomy–AJ	American Institute of Physics	34	1	1957
Avto(mat). svarka	Avtomaticheskaya svarka	Automatic Welding	British Welding Research Association (London)		1	1959
	Avtomatika i Telemekhanika	Automation and Remote Control	Instrument Society of America	27	1	1956
	Biofizika	Biophysics	National Institutes of Health*		1	1957
	Biokhimiya	Biochemistry	Consultants Bureau	21	1	1956
Byull. éksp(erim). biol. i med.	Byulleten' éksperimental'noi biologii i meditsiny	Bulletin of Experimental Biology and Medicine	Consultants Bureau	41	1	1959
DAN (SSSR) } Dokl(ady) AN SSSR }	Doklady Akademii Nauk SSSR	The translation of this journal is published in sections, as follows:				
		Doklady Biochemistry Section	American Institute of Biological Sciences	106	1	1956
		Doklady Biological Sciences Sections (Includes: Anatomy, biophysics, cytology, ecology, embryology, endocrinology, evolutionary morphology, genetics, histology, hydrobiology microbiology, morphology, parasitology, physiology, zoology sections)	American Institute of Biological Sciences	112	1	1957
		Doklady Botanical Sciences Sections (Includes: Botany, phytopathology, plant anatomy, plant ecology, plant embryology, plant physiology, plant morphology sections)	American Institute of Biological Sciences	112	1	1957
		Proceedings of the Academy of Sciences of the USSR, Section: Chemical Technology	Consultants Bureau	106	1	1956
		Proceedings of the Academy of Sciences of the USSR, Section: Chemistry	Consultants Bureau	106	1	1956
		Proceedings of the Academy of Sciences of the USSR, Section: Physical Chemistry	Consultants Bureau	112	1	1957
		Doklady Earth Sciences Sections (Includes: Geochemistry, geology, geophysics, hydrogeology, mineralogy, paleontology, petrography, permafrost sections)	American Geological Institute	124	1	1959
		Proceedings of the Academy of Sciences of the USSR, Section: Geochemistry	Consultants Bureau	106-123	1-6	1957-1958
		Proceedings of the Academy of Sciences of the USSR, Sections: Geology	Consultants Bureau	106-123	1-6	1957-1958
		Doklady Soviet Mathematics	The American Mathematics Society	131	1	1961
		Soviet Physics–Doklady (Includes: Aerodynamics, astronomy, crystallography, cybernetics and control theory, electrical engineering, energetics, fluid mechanics, heat engineering, hydraulics, mathematical physics, mechanics, physics, technical physics, theory of elasticity sections)	American Institute of Physics	106	1	1956
		Proceedings of the Academy of Sciences of the USSR, Applied Physics Sections (does not include mathematical physics or physics sections)	Consultants Bureau	106-117	1	1956-1957
Derevoobrabat. prom-st'.	Derevoobrabatyvayushchaya promyshlennost'	Wood Processing Industry	Timber Development Association (London)		9	1959
	Élecktrosvyaz	Telecommunications	Massachusetts Institute of Technology*		1	1957
Éntom(ol). oboz(renie)	Éntomologicheskoe obozrenie	Entomological Review	American Institute of Biological Sciences	38	1	1959
Farmakol. (i) toksikol(ogiya)	Farmakologiya i toksikologiya	Pharmacology and Toxicology	Consultants Bureau	20	1	1957
FMM	Fizika metallov i metallovedenie	Physics of Metals and Metallography	Acta Metallurgica*	5	1	1957
Fiziol. zhurn. SSSR (im. Sechenova)	Fiziologicheskii zhurnal im. I. M. Sechenova	Sechenov Physiological Journal USSR	National Institutes of Health*		1	1957
Fiziol(ogiya) rast.	Fiziologiya rastenii	Plant Physiology	American Institute of Biological Sciences	4	1	1957
	Geokhimiya	Geochemistry	The Geochemical Society		1	1958
FTT	Fizika tverdogo tela	Soviet Physics–Solid State	American Institute of Physics	1	1	1959
Izmerit. tekh(nika)	Izmeritel'naya tekhnika	Measurement Techniques	Instrument Society of America		1	1959
Izv. AN SSSR, O(td). Kh(im). N(auk)	Izvestiya Akademii Nauk SSSR: Otdelenie khimicheskikh nauk	Bulletin of the Academy of Sciences of the USSR: Division of Chemical Sciences	Consultants Bureau		1	1952

Life Sciences

Chemical Sciences

Earth Sciences

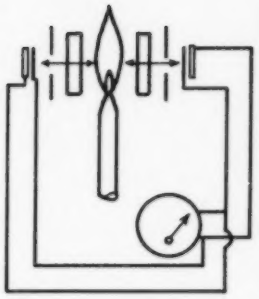
Mathematics

Physics

continued

Izv. AN SSSR, O(td). T(ekhn). N(auk): Met(all). i top.	(see Met. i top.)				
Izv. AN SSSR Ser. fiz(ich).	Izvestiya Akademii Nauk SSSR: Seriya fizicheskaya	Bulletin of the Academy of Sciences of the USSR: Physical Series	Columbia Technical Translations	1	1954
Izv. AN SSSR Ser. geofiz.	Izvestiya Akademii Nauk SSSR: Seriya geofizicheskaya	Bulletin (Izvestiya) of the Academy of Sciences USSR: Geophysics Series	American Geophysical Union	1	1954
Izv. AN SSSR Ser. geol.	Izvestiya Akademii Nauk SSSR: Seriya geologicheskaya	Izvestiya of the Academy of Sciences of the USSR: Geologic Series	American Geological Institute	1	1958
Kauch. i rez.	Kauchuk i rezina	Soviet Rubber Technology	Research Association of British Rubber Manufacturers	18	1 1959
	Kinetika i kataliz	Kinetics and Catalysis	Consultants Bureau	1	3 1960
	Koks i khimiya	Coke and Chemistry USSR	Coal Tar Research Association (Leeds, England)		1 1958
Kolloidn. zh(urn).	Kolloidnyi zhurnal	Colloid Journal	Consultants Bureau	14	1 1952
	Kristallografiya	Soviet Physics – Crystallography	American Institute of Physics	2	1 1957
Metalov. i term. obrabot. metal.	Metallovedenie i termicheskaya obrabotka metallov	Metal Science and Heat Treatment of Metals	Acta Metallurgica	6	1 1958
	Metallurg	Metallurgist	Acta Metallurgica		1 1957
Met. i top.	Metallurgiya i topliva	Russian Metallurgy and Fuels	Eagle Technical Publications		1 1960
Mikrobiol.	Mikrobiologiya	Microbiology	American Institute of Biological Sciences	26	1 1957
OS	Optika i spektroskopiya	Optics and Spectroscopy	American Institute of Physics		6 1959
	Pochvovedenie	Soviet Soil Science	American Institute of Biological Sciences		1 1958
	Priborostroenie	Instrument Construction	British Scientific Instrument Research Association		1 1959
Pribory i tekhn. éks(perimenta)	Pribory i tekhnika éksperimenta	Instruments and Experimental Techniques	Instrument Society of America		1 1957
Prikl. matem. i mekh.	Prikladnaya matematika i mekhanika	Applied Mathematics and Mechanics	American Society of Mechanical Engineers		1 1958
PTÉ	(see Pribory i tekhn. éks.)				
	Problemy Severa	Problems of the North	National Research Council of Canada		
Radiotekh.	Radiotekhnika	Radio Engineering	Massachusetts Institute of Technology*	12	1 1957
Radiotekh. i élektronika	Radiotekhnika i élektronika	Radio Engineering and Electronics	Massachusetts Institute of Technology*	2	1 1957
	Stanki i instrument	Machines and Tooling	Production Engineering Research Assoc.		1 1959
	Stal'	Stal (In English)	Iron and Steel Institute		1 1959
Stek. i keram.	Steklo i keramika	Glass and Ceramics	Consultants Bureau	13	1 1956
Svaroch. proiz-vo	Svarochnoe proizvodstvo	Welding Production	British Welding Research Association		4 1959
Teor. veroyat. i prim.	Teoriya veroyatnostei i ee primenenie	Theory of Probability and Its Applications	Society for Industrial and Applied Mathematics		1 1956
Tsvet. Metally	Tsvetnye metally	Nonferrous Metals	Primary Sources		1 1960
UFN	Uspekhi fizicheskikh Nauk	Soviet Physics – Uspekhi (partial translation)	American Institute of Physics	66	1 1958
UKh	Uspekhi khimii	Russian Chemical Reviews	The Chemical Society (London)		1 1960
UMN	Uspekhi matematicheskikh nauk	Russian Mathematical Surveys	London Mathematical Society	15	1 1960
Usp. fiz. nauk	(see UFN)				
Usp. khim(ii)	(see UKh)				
Usp. matem. nauk	(see UMN)				
Usp. sovr. biol.	Uspekhi sovremennoi biologii	Russian Review of Biology	Oliver and Boyd	48	1959
Vest. mashinostroeniya	Vestnik mashinostroeniya	Russian Engineering Journal	Production Engineering Research Assoc.	4	1959
Vop. gem. i per. krovi	Voprosy gematologii i perelivaniya krovi	Problems of Hematology and Blood Transfusion	National Institutes of Health*	1	1957
Vop. onk.	Voprosy onkologii	Problems of Oncology	National Institutes of Health*	1	1957
Vop. virusol.	Voprosy virusologii	Problems of Virology	National Institutes of Health*	1	1957
Zav(odsk). lab(oratoriya)	Zavodskaya laboratoriya	Industrial Laboratory	Instrument Society of America	25	1 1959
ZhAKh Zh. anal(it). khimii	Zhurnal analiticheskoi khimii	Journal of Analytical Chemistry USSR	Consultants Bureau	7	1 1952
ZhETF Zh. éksperim. i teor. fiz.	Zhurnal éksperimental'noi i theoreticheskoi fiziki	Soviet Physics–JETP	American Institute of Physics	28	1 1955
ZhFKh Zh. fiz. khimii	Zhurnal fizicheskoi khimii	Russian Journal of Physical Chemistry	The Chemical Society (London)		7 1959
ZhMÉI Zh(urn). mikrobiol. épidemiol. i immunobiol.	Zhurnal mikrobiologii, épidemiologii i immunobiologii	Journal of Microbiology, Epidemiology and Immunobiology	National Institutes of Health*		1 1957
ZhNKh Zh(urn). neorgan(ich). khim(ii)	Zhurnal neorganicheskoi khimii	The Russian Journal of Inorganic Chemistry	The Chemical Society (London)		1 1959
ZhOKh Zh(urn). obshch(ei) khimii	Zhurnal obshchei khimii	Journal of General Chemistry USSR	Consultants Bureau	19	1 1949
ZhPKh Zh(urn). prikl. khimii	Zhurnal prikladnoi khimli	Journal of Applied Chemistry USSR	Consultants Bureau	23	1 1950
ZhSKh Zh(urn). strukt. khimii	Zhurnal strukturnoi khimii	Journal of Structural Chemistry	Consultants Bureau	1	1 1960
ZhTF Zh(urn). tekhn. fiz.	Zhurnal teknicheskoi fiziki	Soviet Physics–Technical Physics	American Institute of Physics	26	1 1956
Zh(urn). vyssh. nervn. deyat. (im. Pavlova)	Zhurnal vysshei nervnoi deyatel'nosti (im. I. P. Pavlova)	Pavlov Journal of Higher Nervous Activity	National Institutes of Health*		1 1958

*Sponsoring organization. Translation through 1960 issues is a publication of Pergamon Press.



TECHNIQUES IN FLAME PHOTOMETRIC ANALYSIS

by N. S. Poluéktov

TRANSLATED FROM RUSSIAN

Original published by the
State Scientific-Technical Press
for Chemical Literature, Moscow

This volume contains a practical and comprehensive survey of the techniques employed and the instruments required for this important rapid analysis method, as well as a brief account of the theoretical principles involved. Experimental procedures and the design of apparatus are discussed at length, making the relevant chapters a valuable manual for all chemists concerned with analytical problems.

The second half of the book deals with the determination of individual elements in a variety of materials, providing much factual information, together with a complete and international bibliography. There are many useful tables, and two appendices which consider wavelengths of brightest spectral lines and peaks of molecular bands of elements excited in flame, and recommended wavelengths of spectral lines and peaks of molecular bands for determination of elements with the aid of a spectrophotometer employing glass optics and using an air-acetylene flame.

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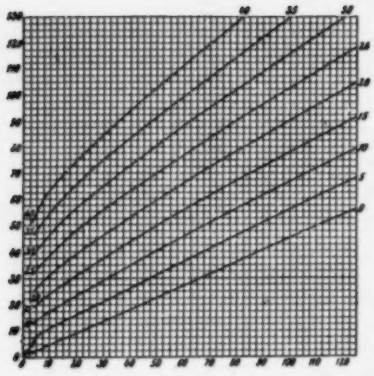

TABLES AND NOMOGRAMS OF HYDROCHEMICAL ANALYSIS

by I. Yu. Sokolov

Translated from Russian

CONSULTANTS BUREAU
227 W. 17 ST., NEW YORK 11, N. Y.

Upon completion of analytical experiments, every research chemist, whether he uses a "test tube" or a "пробирка," must calculate his findings in the international language of figures in two forms (weights and equivalents), and often three (weights, equivalents and percent-equivalents), and then compare experimental data with theoretical values.

Technicians and statisticians working with the results of hydrochemical analyses performed at different times by different laboratories inevitably face the problem of converting their figures to one system.

All such calculations are considerably simplified by the use of the tables and nomograms in this book, originally published by the State Scientific and Technical Press for Literature on Geology and the Conservation of Mineral Resources, Moscow.

All the tables and nomograms are based on analytical results expressed in the form widely used in hydrogeological practice—milligrams per liter (weight form) and milligram-equivalents per liter (equivalent form). For calculation of percent-equivalents, the sum of cation equivalents and the sum of anion equivalents are taken as 100% each. Several new tables are presented for the first time, and the many tables for converting water-analysis results from one form to another make it possible to find the milligram-equivalents for any practically possible content of a component in water, accurate to the second decimal place, and the weight content of substances to tenths of a milligram.

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Introduction
Tables for converting water-analysis results from one form to another
- I. Table for converting hardness, expressed in German degrees, into milligram-equivalents
- II. Table for converting milligram-equivalents of Ca^{2+}, Mg^{2+} and HCO_3^- into German degrees of hardness
- III. Tables for converting milligrams into milligram-equivalents
- IV. Tables for converting milligram-equivalents into milligrams
- V. Tables for converting oxides to ions
- VI. Tables for converting nitrogen into nitrogen-containing ions
- VII. Table for converting milligrams of NH_3 into milligrams of NH_4^+
- VIII. Table for converting oxidizability with milligrams of $KMnO_4$ into milligrams of O

Factors for converting the results of water analysis from one form into another
Average value of activity coefficient f_{av}
Nomogram for calculating percent-equivalents
Nomogram for calculating pH from given values of free CO_2 and HCO_3^-
Calculation of aggressive carbon dioxide
Relation between different forms of weak acids in natural waters at different pH values and ionic strengths μ
Factors for converting different forms of expressing aqueous solution concentrations into milligrams and milligram-equivalents
International atomic weights (Appendix)

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